

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Peter S. Linsley, et al.

Serial No. : 09/609,915

Examiner : Lorraine Spector, Ph.D.

Filed: July 3, 2000

Group Unit : 1646

For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

55 S. Lake Ave., Suite 710
Pasadena, California 91101
November 28, 2005

MAIL STOP PETITIONS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir/Madam:

PETITION UNDER 37 C.F.R. §1.183

By way of this Petition, Applicants request that the Patent Office accept the attached Written Consent of Assignee under 37 C.F.R. §3.73(b) ("Rule 73(b)") and waive the requirement of Rule 73(b) that ownership be established by documentary evidence of a chain of title from the original owner (i.e., inventor: Jeffrey A. Ledbetter) to assignee in the subject application. Specifically, in the subject application, all inventors, except Dr. Jeffrey A. Ledbetter, executed Assignments of Rights to Bristol-Myers Squibb Company. Dr. Ledbetter, who also refuses to execute the Declaration that is necessary to file a Request to Correct the Inventorship Under 37 C.F.R. §1.48(a), in connection with the above-referenced patent application, refuses to execute an Assignment of Rights to Bristol-Myers Squibb Company in the subject application despite being under contractual obligation to do so.

However, Dr. Ledbetter has executed Assignments of Rights to Bristol-Myers Squibb Company for all the patent applications in the chain of title of the subject application which are recorded at the U.S. Patent and Trademark Office at:

BEST AVAILABLE COPY

- Reel 7169, Frame 0094, for U.S. Serial No. 08/228,208
- Reel 6567, Frame 0839, for U.S. Serial No. 08/008,898
- Reel 5821, Frame 588, for U.S. Serial No. 07/723,617

Given Dr. Ledbetter's previous assignments and that his contributions to the claimed invention are disclosed in the parent applications to which the subject application claims priority and which parent applications he has assigned to Bristol-Myers, Applicants respectfully request suspension of rules involving Rule 73(b) establishing right of assignee to take action with regard to Dr. Ledbetter's lack of Assignment in the subject application.

Applicants submit herewith the fee under 37 C.F.R. §1.17(h) of \$130.00 in compliance with 37 C.F.R. § 1.183.

No fee, other than the fee under 37 C.F.R. §1.17(h), is deemed necessary in connection with the filing of this Petition. If any additional fees are necessary, the Patent Office is authorized to charge any additional fees to Deposit Account No. 50-0306.

Respectfully submitted,



Sarah B. Adriano
Registration No. 34,470
SaraLynn Mandel
Registration No. 31,853
Attorneys for Applicants
Mandel & Adriano
55 S. Lake Ave., Suite 710
Pasadena, California 91101
(626) 395-7801
Customer No. 26,941

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley, et al.
Serial No. : 09/609,915 Examiner: Lorraine Spector, Ph.D.
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For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES
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55 S. Lake Ave., Suite 710
Pasadena, California 91101
November 28, 2005

MAIL STOP PETITIONS

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

SIR/MADAM:

**REQUEST TO CORRECT THE INVENTORSHIP
UNDER 37 C.F.R. §1.48(a),
PETITION UNDER 37 C.F.R. §1.47(a), AND
PETITION UNDER 37 C.F.R. §1.183**

Applicants submit this request to correct the inventorship in the above-identified patent application under 37 C.F.R. §1.48(a). In order to correct the inventorship, Applicants also submit a (1) Petition under 37 C.F.R. §1.47(a) to request that the U. S. Patent and Trademark Office accept the enclosed Combined Declarations and Powers of Attorney under 37 C.F.R. §1.63 executed by co-inventors Peter S. Linsley, Jurgen Bajorath, Robert J. Peach, William Brady and Nitin Damle, on behalf of themselves, and the only non-signing inventor, Jeffrey A. Ledbetter (Exhibit 5A); and (2) Petition under 37 C.F.R. §1.183 to suspend the rules regarding 37 C.F.R. §3.73(b) that ownership be established by documentary evidence of a chain of title from the original inventor to assignee in the subject application and to accept the attached Written Consent of Assignee under 37

C.F.R. §3.73(b) ("Rule 73(b)") despite the lack of an executed assignment from Dr. Ledbetter to Bristol-Myers Squibb Company in the subject application (Exhibit 3).

The inventive entity, Peter S. Linsley, Jeffrey A. Ledbetter, Jurgen Bajorath, Robert J. Peach, William Brady, Philip Wallace, and Nitin Damle, is set forth, in error, in the executed Declaration Under 37 C.F.R. §1.63, filed on February 28, 2001, with the U.S. Patent and Trademark Office, in connection with the above-referenced application. This inadvertent error arose without any deceptive intent by the named inventors.

Peter S. Linsley, Jeffrey A. Ledbetter, Jurgen Bajorath, Robert J. Peach, William Brady, Philip Wallace, and Nitin Damle, were originally named as the inventors of the originally filed claims of the subject application.

The subject application was originally filed with claims 1-25. As part of a response to a restriction requirement, Applicants added (a) claims 26-28, on June 2, 2004; and (b) claims 29-30, on August 2, 2004. Further, on August 2, 2004, Applicants elected, with traverse, the invention of claims 29-30 for prosecution on the record. Claims 1-2, 5, 9 and 11-30 are pending, although only claims 29-30 are being examined.

Upon review, Philip Wallace is not an inventor of the originally filed claims 1-25 (which are deemed withdrawn but currently pending), later filed claims 26-28 (which are deemed withdrawn but currently pending), or later filed and elected, pending claims 29-30. Therefore, Applicants respectfully request that Philip Wallace be removed as an inventor of the pending claims of the subject application.

The correct inventive entity of the originally filed claims of the subject application *is* Peter S. Linsley, Jeffrey A. Ledbetter, Jurgen Bajorath, Robert J. Peach, William Brady, and Nitin Damle.

In accordance with 37 C.F.R. §1.48(a), in addition to this Request, Applicants also submit:

1. A Declaration from Dr. Philip Wallace, who is being deleted as an inventor and stating that the error in inventorship occurred without deceptive intention on his part (Exhibit 4B);
2. Declarations under 37 C.F.R. §1.63 executed by the actual inventors except for inventor Ledbetter (Exhibit 4C);
3. A copy of a Written Consent of Assignee (Exhibit 4D); and
4. A fee of \$130.00 under 37 C.F.R. §1.17(i).

Since Dr. Ledbetter refuses to sign the Declaration under 37 C.F.R. §1.63, in accordance with the requirements under 37 C.F.R. §1.47(a), Applicants provide the following:

1. Petition Under 37 C.F.R. §1.47 (a) (Exhibit 5A);
2. Copies of Combined Declarations and Powers of Attorney under 37 C.F.R. §1.63 executed by co-inventors Peter S. Linsley, Jurgen Bajorath, Robert J. Peach, William Brady and Nitin Damle (Exhibit 5B);
3. Copy of a letter sent to the last known address of a non-signing inventor, Jeffrey A. Ledbetter, including enclosures: (1) an original Combined Declaration and Power of Attorney; (2) copy of amendments filed with the application; (3) copy of the originally filed patent application; and (4) copy of pending claims 1-2, 5, 9, 11-30 (Exhibit 5C); and
4. Copy of the Federal Express email confirmation and delivery information indicating that the letter was signed for in behalf of Jeffrey A. Ledbetter (Exhibit 5D); and
5. A fee of \$130.00 under 37 C.F.R. §1.17(h).

Additionally, Applicants wish to note that this Petition under 37 C.F.R. §1.47(a) is the second such Petition being filed. The first Petition under 37 C.F.R. §1.47(a) was filed in the subject application on February 28, 2001 and submitted for reconsideration on November 5, 2001 (although date-stamped received by the Patent Office on January 4, 2002) because at that time, Dr. Ledbetter also refused to sign the Declaration under 37 C.F.R. §1.63 as part of the originally filed subject nonprovisional application. The Patent Office granted Applicants' earlier filed Petition under 37 C.F.R. §1.47(a), and for convenience, copies of the Decision Granting Status Under 37 C.F.R. §1.47(a) and a letter from the U.S. Patent Office addressed to inventor Ledbetter, both dated January 25, 2002 are attached (Exhibit 5E).

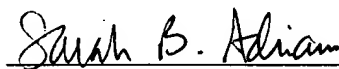
Further, since Dr. Ledbetter also refuses to execute an Assignment of Rights in the subject application to Bristol-Myers Squibb Company, in accordance with the requirements under 37 C.F.R. §1.183, Applicants provide:

1. a Petition under 37 C.F.R. §1.183 to suspend the rules regarding 37 C.F.R. §3.73(b) that ownership be established by documentary evidence of a chain of title from the original inventor to assignee in the subject application and to accept the attached Written Consent of Assignee under 37 C.F.R. §3.73(b) ("Rule 73(b)") despite the lack of an executed assignment from Dr. Ledbetter to Bristol-Myers Squibb Company (Exhibit 3) in the subject application; and
2. A fee of \$130.00 under 37 C.F.R. §1.17(h).

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Filed: July 3, 2000
Page 5

No fee, other than the above-mentioned fees, is deemed necessary in connection with the filing of this Request. If any further fees are necessary, the Patent Office is authorized to charge the additional fees to Deposit Account No. 50-0306.

Respectfully submitted,



Sarah B. Adriano
Registration No. 34,470
SaraLynn Mandel
Registration No. 31,853
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55 South Lake Ave., Suite 710
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(626) 395-7801
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley, et al.

Serial No. : 09/609,915

Examiner : Lorraine Spector, Ph.D.

Filed: : July 3, 2000

Group Unit : 1646

For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES
THEREOF

55 S. Lake Ave., Suite 710
Pasadena, California 91101
November 28, 2005

MAIL STOP PETITIONS
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir/Madam:

PETITION UNDER 37 C.F.R. §1.47(a)

Dr. Jeffrey A. Ledbetter refuses to sign the Declaration that is necessary to file a Request to Correct the Inventorship Under 37 C.F.R. §1.48(a), in connection with the above-referenced patent application.

Accordingly, Applicants submit this Petition under 37 C.F.R. §1.47(a) and request that the U. S. Patent and Trademark Office accept the enclosed Combined Declarations and Powers of Attorney executed by co-inventors Peter S. Linsley, Jurgen Bajorath, Robert J. Peach, William Brady and Nitin Damle, on behalf of themselves and the only non-signing inventor, Jeffrey A. Ledbetter. The executed Declarations by Drs. Peter S. Linsley, Jurgen Bajorath, Robert J. Peach, William Brady and Nitin Damle are attached herewith as **Exhibit 5B**.

The facts are as follows:

The last known residence address for only non-signing inventor Ledbetter is as follows:

Dr. Jeffrey A. Ledbetter
306 N.W. 113th Place
Seattle, Washington 98117

On December 23, 2004, Applicants' undersigned attorney sent a letter to Dr. Jeffrey A. Ledbetter enclosing (1) an original Combined Declaration and Power of Attorney under 37 C.F.R. §1.63, (2) copies of Amendments filed in connection with the subject application, and (3) a copy of the originally-filed patent application, and requesting that Dr. Ledbetter execute and return the Combined Declaration and Power of Attorney under 37 C.F.R. §1.63 (**Exhibit 5C**).

The attached Federal Express email confirmation and delivery information sheet provides confirmation that the December 23, 2004 letter and enclosures were delivered and received (**Exhibit 5D**).

As of the filing date of this Petition, inventor Ledbetter has not returned an executed Declaration. The fact that no executed Declaration was returned to Applicants' undersigned attorney within the requested time, is deemed a refusal to execute the Declaration under 37 C.F.R. §1.63 by the only remaining non-signing inventor Ledbetter.


Applicants wish to note that this Petition under 37 C.F.R. §1.47(a) is the second such Petition being filed. The first Petition under 37 C.F.R. §1.47(a) was filed in the subject application on February 28, 2001 and submitted for reconsideration on November 5, 2001 (although date stamped received by the Patent Office on January 4, 2002). At that time, Dr. Ledbetter also refused to sign the Declaration under 37 C.F.R. §1.63 as part of the originally filed subject nonprovisional application. The Patent Office granted

Applicants' earlier filed Petition under 37 C.F.R. §1.47(a) and a copy of the Decision Granting Status Under 37 C.F.R. §1.47(a) and a letter from the U.S. Patent Office addressed to inventor Ledbetter, both dated January 25, 2002 is attached (**Exhibit 5E**).

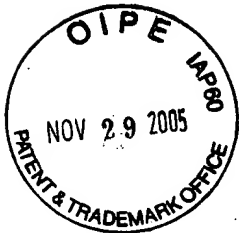
Applicants submit herewith the fee under 37 C.F.R. §1.17(h) of \$130.00 in compliance with 37 C.F.R. § 1.47(a).

No fee, other than the fee under 37 C.F.R. §1.17(h), is deemed necessary in connection with the filing of this Petition. If any additional fees are necessary, the Patent Office is authorized to charge any additional fees to Deposit Account No. 50-0306.

Respectfully submitted,



Sarah B. Adriano
Registration No. 34,470
SaraLynn Mandel
Registration No. 31,853
Patent Practitioners for Applicants
Mandel & Adriano
55 S. Lake Ave., Suite 710
Pasadena, California 91101
(626) 395-7801
Customer No. 26,941



Dkt.30436.30USI2/SBA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley et al.
Serial No : 09/609,915 Examiner: Lorraine Spector, Ph.D.
Filed : July 3, 2000 Group Art Unit: 1647
For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES
THEREOF

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

SIR:

**TERMINAL DISCLAIMER TO OBVIATE
A DOUBLE PATENTING REJECTION**

Dear Sir:

Petitioner, The Bristol-Myers Squibb Company, a corporation organized and existing under the laws of the State of New Jersey and having its primary place of business at P.O. Box 4000, Lawrenceville-Princeton Road, Princeton, New Jersey 08453-4000, in the county of Mercer, represents that it is the owner of the entire right, title and interest in the following U.S. Patents:

- (1) U.S. Patent No. 5,844,095, issued on December 1, 1998 and entitled CTLA4 Ig FUSION PROTEINS, at Reel 8733, Frame 0034 and Reel 8788, Frame 0644.
- (2) U.S. Patent No. 5,851,795, issued on December 22, 1998 and entitled SOLUBLE CTLA4 MOLECULES AND USES THEREOF at Reel 8733, Frame 0034 and Reel 8788, Frame 0644.
- (3) U.S. Patent No. 5,885,796, issued on March 23, 1999 and entitled CTLA4 RECEPTOR AND USES THEREOF, at Reel 8733, Frame 0034 and Reel 8788, Frame 0644.

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Filed: July 3, 2000
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Attached herewith is a corresponding Certificate under 37 C.F.R. § 3.73(b) establishing Bristol-Myers Squibb Company right as assignee to take action (EXHIBIT 2).

Petitioner, Bristol-Myers Squibb Company hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the above-identified application, which would extend beyond the expiration date of the full statutory term of U.S. Patents:

- (1) U.S. Patent No. 5,844,095, issued on December 1, 1998;
- (2) U.S. Patent No. 5,851,795, issued on December 22, 1998;
- (3) U.S. Patent No. 5,885,796, issued on March 23, 1999;

and hereby agrees that any patent so granted on the above-identified application shall be enforceable only for and during such period that the legal title to said patent shall be the same as the legal title to U.S. Patent No. 5,844,095; 5,851,795 and 5,885,796 respectively, this agreement to run with any patent granted on the above-identified application and to be binding upon the grantee, its successors, or assigns.

In making the above disclaimer, Petitioner does not disclaim the terminal part of any patent granted on the above-identified application that would extend to the full statutory term as presently shortened by any terminal disclaimer of U.S. Patent No. 5,844,095; 5,851,795 and 5,885,796 respectively, in the event that any such issued patent: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid, is statutorily disclaimed in whole or terminally disclaimed under 37 C.F.R. §1.321(a), has all claims cancelled by a reexamination certification, or is otherwise terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer, except for the separation of legal title stated above.

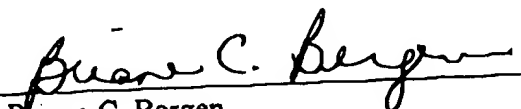
Peter S. Linsley et al
U. S. Serial No. 09/609,915
Filed: July 3, 2000
Page 3

For submissions on behalf of an organization (e.g. corporation, partnership, university, government agency, etc.), the undersigned (whose title is supplied below) is empowered to act on behalf of the organization.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: _____

11/28/05



Briana C. Bergen
Registration No. 39,123
Senior Counsel - Biotechnology Patents
Biotechnology Patents
Bristol-Myers Squibb Company
P.O. Box 4000
Princeton, New Jersey 08543-4000
Customer No. 23,914

THE STATEMENT BELOW IS FOR OFFICE USE ONLY

In accordance with the decision granting the petition filed on _____, 2005,
this terminal disclaimer is accepted. The period of patent to lapse specified above has
been accepted as equivalent to _____ months.

Petitions Examiner



DKT. 30436.30USI2/SBA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley, Jeffrey A. Ledbetter, Jorgen Bajorath, Robert J. Peach, William Brady, and Nitin Damle

Serial No. : 09/609,915 Examiner: Lorraine Spector, Ph.D.

Filed : July 3, 2000 Group Art Unit: 1647

Title : SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir/Madam:

WRITTEN CONSENT OF ASSIGNEE UNDER 37 C.F.R. §3.73(b)

Petitioner, the Bristol-Myers Squibb Company, a corporation organized and existing under the laws of the State of New Jersey and having its primary place of business at P.O. Box 4000, Lawrenceville-Princeton Road, Princeton, New Jersey 08453-4000, certifies that it is the assignee of the entire right, title and interest in the patent application identified above by virtue of assignments from the inventors for the subject application and related parent patent applications.

The subject application is: a continuation-in-part of U.S. Serial No. 08/228,208, filed April 15, 1994, now U.S. Patent No. 6,090,914, issued July 18, 2000, which was a continuation-in-part of U.S. Serial No. 08/008,898, filed January 22, 1993, now U.S. Patent No. 5,770,197, issued June 23, 1998, which was a continuation-in-part of U.S. Serial No. 07/723,617, filed June 27, 1991, now abandoned.

Applicants: Peter S. Linsley, et al.
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Filed: July 3, 2000
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The assignment for the parent patent applications in the chain of title for the subject application U.S. Serial No. 09/609,915 are recorded at the U.S. Patent and Trademark Office as follows:

- Reel 7169, Frame 0094, for U.S. Serial No. 08/228,208 (Exhibit 2A) except for the recorded assignments of Drs. Peach and Bajorath (attached herewith as Exhibit 2A-1 including the recordation form cover sheet)
- Reel 6567, Frame 0839, for U.S. Serial No. 08/008,898 (Exhibit 2B)
- Reel 5821, Frame 588, for U.S. Serial No. 07/723,617 (Exhibit 2C)

The assignments for the subject application have been executed by all the inventors except for Dr. Jeffrey Ledbetter (see attached as Exhibit 2D).

The undersigned has reviewed all the documents in the chain of title of the patent application identified above. Despite Dr. Ledbetter's refusal to execute an Assignment of Rights from himself to Bristol-Myers Squibb Company in the subject application and because Dr. Ledbetter's contributions to the claimed invention are disclosed in the parent applications to which the subject application claims priority and which parent applications Dr. Ledbetter has assigned to Bristol-Myers Squibb Company, to the best of undersigned's knowledge and belief, title is in the assignee identified above. However, in case the Patent Office disagrees, Applicants provide a Petition under 37 C.F.R. §1.183 requesting that the Patent Office suspend the rules regarding 37 C.F.R. §3.73(b) that ownership be established by documentary evidence of a chain of title from the original inventor (i.e. inventor Ledbetter) to assignee in the subject application and to accept the Written Consent of Assignee under 37 C.F.R. §3.73(b) (Exhibit 3).

The undersigned (whose title is provided below) is empowered to act on behalf of the assignee. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and

Applicants: Peter S. Linsley, et al.

U.S. Serial No: 09/609,915

Filed: July 3, 2000

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further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 11/28/05

By:

Briana C. Bergen

Briana C. Bergen

Registration No. 39,123

Senior Counsel - Biotechnology Patents

Biotechnology Patents

Bristol-Myers Squibb Company

P.O. Box 4000

Princeton, New Jersey 08543-4000

Customer No. 23,914



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PAGE: 1

PATENT NUMBER: 6090314
SERIAL NUMBER: 08/228208
RELATED PATENT NUMBERS: 5773253 5844095 5851795 5885796
TITLE: CTLA4/CD28IG HYBRID FUSION PROTEINS AND USES THEREOF
APPLICANT: LINSLEY, PETER S. ; LEDBETTER, JEFFREY A.
BAJORATH, JURGEN ; PEACH, ROBERT ; BRADY, WILLIAM

REEL: 007169 FRAME: 0094 DATE RECORDED: 10/20/94 NUMBER OF PAGES: 008
ASSIGNOR: LINSLEY, PETER S.

EXC DATE: 09/30/94
LEDBETTER, JEFFREY A.
EXC DATE: 09/29/94
DAMLE, NITIN K.
EXC DATE: 07/01/94
BRADY, WILLIAM
EXC DATE: 09/29/94

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PAGE: 2

WALLACE, PHILIP M.
EXC DATE: 09/29/94
ASSIGNEE: BRISTOL-MYERS SQUIBB COMPANY
3005 FIRST AVENUE
SEATTLE, WA 98121
BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).
RETURN ADDRESS: SARAH B. ADRIANO, ESQ.
MERCHANT, GOULD, SMITH, EDELL, ET AL.
11150 SANTA MONICA BLVD.
LOS ANGELES, CA 90025-3395

NO MORE INFORMATION FOR THIS PATENT NUMBER 10/11/01 15:02



RECORDATION FORM COVER SHEET
PATENTS ONLY

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

To the Honorable Commissioner of Patents and Trademarks: Please record the attached original documents or copy thereof.

1. Name of conveying party(ies):

Peter S. Linsley
Jeffrey A. Ledbetter
Nitin K. Dangle
William Brady
Philip M. Wallace

2. Name and address of receiving party(ies):

Name: Brigol-Myers Squibb Company

Internal Address: _____

Additional name(s) of conveying party not attached? (Yes/No)

3. Nature of conveyance:

- ☒ Assignment ☐ Merger
☐ Security Agreement ☐ Change of Name
☐ Other _____

Street Address: 1005 First Avenue

City: Seattle State: WA ZIP: 98121

Execution Date: July 1, 1994; Sept. 29, 1994; Sept. 30, 1994

Additional name(s) & address(es) attached? (Yes/No)

4. Application number(s) or patent number(s):

If this document is being filed together with a new application, the execution date of the application is: _____

A. Patent Application No(s)
08/228,308

B. Patent No(s)

Additional numbers attached? (Yes/No)

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: Sarah B. Adriano, Esq.
Address: Merchant, Gould, Smith, Edell,
Welter & Schmidt
Suite 400
1150 Santa Monica Boulevard
Los Angeles, CA 90025-3395

6. Total number of applications and patents involved: _____

7. Total fee (37 CFR 1.31): \$ 40.00

- ☒ Enclosed
☐ Authorized to be charged to deposit account

8. Please charge any additional fees or credit any overpayments to our Deposit account number: 12-2724

(Duplicate copy of this page is attached.)

UPV KA 10/28/94 08228208

NO PAY FOR THIS SHEET
1 \$B1 40.00 CK

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9. Document and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and my attached copy is a true copy of the original document.

Sarah B. Adriano, Esq.
Name of Person Signing

Sarah B. Adriano
Signature

October 17, 1994
Date

Total number of pages including cover sheet, attachments, and documents: 5

(Pub. No. 3451-1011 (imp. 4/94))

Do not detach this portion

Note: Documents to be recorded with required cover sheet information are:

Commissioner of Patents and Trademarks
Patent and Trademark Office
Washington, D.C. 20231

Public burden reporting for this sample cover sheet is estimated to average about 10 minutes per document to be recorded, including time for reviewing the document and gathering the data needed, and completion and reviewing the sample cover sheet. Send your comments regarding this burden estimate to the U.S. Patent and Trademark Office, Office of Information Systems, P.O. Box 10, Washington, D.C. 20231, and to the Office of Management and Budget, Paperwork Reduction Project (0651-0011), Washington, D.C. 20503. DO NOT SEND COMPLETED COVER SHEETS WITH ASSIGNMENTS TO THIS ADDRESS.

ASSIGNMENT

WHEREAS, Peter S. Linsley, residing at 2430 9th Avenue West, Seattle, WA 98119; Jeffrey A. Ledbetter, residing at 306 N.W. 113th Place, Seattle, WA 98117; Nitin K. Damle, residing at 865 Ridge Road, Monmouth Junction, NJ 08852; William Brady, residing at 618 219th Place S.W., Bothell, WA 98021; and Philip M. Wallace, residing at 3020 64th Avenue Southwest, #D, Seattle, WA 98116 (hereinafter referred to as "Assignors"), made certain new and useful inventions and improvements for which they executed an application for Letters Patent of the United States which is entitled CTIA4 MOLECULES AND IL4-BINDING MOLECULES AND USES THEREOF, (U.S. Serial No. 08/228,208, filed April 15, 1994).

AND WHEREAS, BRISTOL-MYERS SQUIBB COMPANY (hereinafter referred to as "Assignee"), a corporation organized and existing under and by virtue of the laws of the State of Delaware and having an office and place of business at 3005 First Avenue, Seattle, WA 98121, is desirous of acquiring the right, title and interest in and to said inventions, improvements and application and in and to the Letters Patent to be obtained thereof;

NOW, THEREFORE, to all whom it may concern, be it known that for and in consideration of the sum of One Dollar and other good and valuable considerations, the receipt and sufficiency whereof is hereby acknowledged, have sold, assigned and transferred, and by these presents do sell, assign and transfer unto said Bristol-Myers Squibb Company, its successors or assigns, the entire right title and interest for all countries in and to all inventions and improvement disclosed in the aforesaid application, and in and to the said application, all divisions, continuations, or renewals thereof, all Letters Patent which may be granted therefrom, and all reissues or extensions of such patents, and in and to any and all applications which have been or shall be filed in any foreign countries for Letters Patent on the said inventions and improvements, including an assignment of all rights under the provisions of the International Convention, and all Letters Patent of foreign countries which may be granted therefrom; and Peter S. Linsley, Jeffrey A. Ledbetter, Nitin K. Damle, William Brady and Philip M. Wallace do hereby authorize and request the Commissioner of Patents to issue any and all United States Patent for the aforesaid inventions and improvements to the said Bristol-Myers Squibb Company as the assignee of the entire right, title and interest in and to the same, for the use of the said Bristol-Myers Squibb Company, its successors and assigns.

REEL 1169 FRAME 95

AND, for the consideration aforesaid, Peter S. Linsley, Jeffrey A. Ledbetter, Nitin K. Damle, William Brady and Philip M. Wallace do hereby agree that Peter S. Linsley, Jeffrey A. Ledbetter, Nitin K. Damle, William Brady and Philip M. Wallace, their executors and legal representatives will make, execute and deliver any and all other instruments in writing including any and all further application papers, affidavits, assignments and other documents, and will communicate to said Bristol-Myers Squibb Company, its successors and representatives all facts known to us relating to said improvements and the history thereof and will testify in all legal proceedings and generally do all things which may be necessary or desirable more effectually to secure to and vest in Bristol-Myers Squibb Company, its successors or assigns the entire right, title and interest in and to the said improvements, inventions, applications, Letters Patent, rights, titles, benefits, privileges and advantages hereby sold, assigned and conveyed, or intended so to be.

AND, furthermore, Peter S. Linsley, Jeffrey A. Ledbetter, Nitin K. Damle, William Brady and Philip M. Wallace covenant and agree with said Bristol-Myers Squibb Company its successors and assigns, that no assignment, grant, mortgage, license or other agreement affecting the rights and property herein conveyed has been made to others by Peter S. Linsley, Jeffrey A. Ledbetter, Nitin K. Damle, William Brady, and Philip M. Wallace, and that full right to convey the same as herein expressed is possessed by Peter S. Linsley, Jeffrey A. Ledbetter, Nitin K. Damle, William Brady and Philip M. Wallace.

WELT 169 FRAT-98

Jeffrey A. Ledbetter
Jeffrey A. Ledbetter

STATE OF Washington)
COUNTY OF King) ss.

On this 29 day of September, 1994, before me,
Jessie Kay Bule, personally appeared Jeffrey A. Ledbetter,
personally known to me or (proved to me on the basis of
satisfactory evidence to be the person(s)), whose name(s) is/are
subscribed to the within instrument and acknowledged to me that
he/she executed the same in his/her authorized capacity(ies), and
that by his/her signature(s) on the instrument the person(s), or
the entity upon behalf of which the person(s) acted, executed the
instrument.

WITNESS my hand and official seal.

Jessie Kay Bule
Notary

REEL 1104 FRANK 0-474

William Brady
William Brady

STATE OF Washington)
COUNTY OF King) ss.

On this 29th day of September, 1994, before me,
Joi Kay Butler, personally appeared William Brady,
personally known to me or (proved to me on the basis of
satisfactory evidence to be the person(s)) whose name(s) is/are
subscribed to the within instrument and acknowledged to me that
he/she executed the same in his/her authorized capacity(ies), and
that by his/her signature(s) on the instrument the person(s), or
the entity upon behalf of which the person(s) acted, executed the
instrument.

WITNESS my hand and official seal.

Joi Kay Butler
Notary

REEL 1169 FRANKS 98

P. Wallace
Philip M. Wallace

STATE OF Washington)
COUNTY OF King) ss.

On this 29th day of September, 1994, before me, Lrs. Kay Bulen, personally appeared Philip M. Wallace, personally known to me or (proved to me on the basis of satisfactory evidence to be the person(s)) whose name(s) is/are subscribed to the within instrument and acknowledged to me that he/she executed the same in his/her authorized capacity(ies), and that by his/her signature(s) on the instrument the person(s), or the entity upon behalf of which the person(s) acted, executed the instrument.

WITNESS my hand and official seal.

Lrs. Kay Bulen
Notary

REEL 1104 FRANKO 74


Nitin K. Damle

STATE OF N.J.)
COUNTY OF Middlesex) ss.

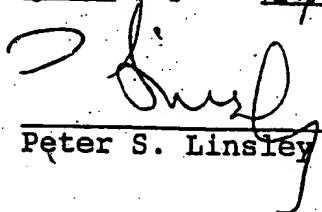
On this 1st day of July, 1994, before me,
Nitin K. Damle, personally appeared _____,
personally known to me or (proved to me on the basis of
satisfactory evidence to be the person(s)) whose name(s) is/are
subscribed to the within instrument and acknowledged to me that
he/she executed the same in his/her authorized capacity(ies), and
that by his/her signature(s) on the instrument the person(s), or
the entity upon behalf of which the person(s) acted, executed the
instrument.

WITNESS my hand and official seal.

Marie Arnes
Notary

REC 7169 FRANK 100

IN TESTIMONY WHEREOF, Peter S. Linsley, Jeffrey A. Ledbetter, Nitin K. Damle, William Brady, and Philip M. Wallace have hereunto set their hands this 30th day of September, 1994.


Peter S. Linsley

STATE OF Washington)
COUNTY OF Finn) ss.

On this 30th day of September, 1994, before me, Mrs Kay Bulmer, personally appeared Peter S. Linsley, personally known to me or (proved to me on the basis of satisfactory evidence to be the person(s)) whose name(s) is/are subscribed to the within instrument and acknowledged to me that he/she executed the same in his/her authorized capacity(ies), and that by his/her signature(s) on the instrument the person(s), or the entity upon behalf of which the person(s) acted, executed the instrument.

WITNESS my hand and official seal.

Mrs Kay Bulmer
Notary

RECORDED
PATENT & TRADEMARK OFFICE

OCT 20 94



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Peter S. Linsley et al.
Patent No.: 6,090,914
Issued: July 18, 2000
Docket: 30436.30US01
Title: CTLA4/CD28IG HYBRID FUSION PROTEINS AND USES THEREOF

CERTIFICATE UNDER 37 CFR 1.8

I hereby certify that this paper or fee is being deposited with the United States Postal Service as first class mail in an envelope addressed to the Mail Stop: Assignment Recordation Services, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313 on August 12, 2005.

By: 
Name: Richelle Ann Dorringer

55 S. Lake Avenue, Suite 710
Pasadena, California 91101
August 12, 2005

Mail Stop ASSIGNMENT RECORDATION SERVICES
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

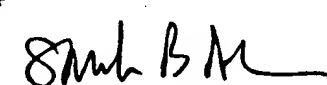
Sir:

We are transmitting herewith the attached:

- ☒ Transmittal sheet, in duplicate, containing Certificate under 37 CFR 1.8.
- ☒ Recordation Form Cover Sheet (1 page)
- ☒ Assignment (4 pages)
- ☒ A check in the amount of \$40.00 to cover the fee
- ☒ Return postcard

Please charge any additional fees or credit overpayment to Deposit Account No. 50-0306. A duplicate of this sheet is enclosed.

MANDEL & ADRIANO
55 S. Lake Avenue, Suite 710
Pasadena, California 91101
(626)395-7801

By: 
Name: Sarah B. Adriano
Reg. No.: 34,470
Initials: SBA

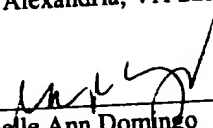


IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Peter S. Linsley et al.
Patent No.: 6,090,914
Issued: July 18, 2000
Docket: 30436.30US01
Title: CTLA4/CD28IG HYBRID FUSION PROTEINS AND USES THEREOF

CERTIFICATE UNDER 37 CFR 1.8

I hereby certify that this paper or fee is being deposited with the United States Postal Service as first class mail in an envelope addressed to the Mail Stop: Assignment Recordation Services, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313 on August 12, 2005.

By: 
Name: Richelle Ann Domingo

55 S. Lake Avenue, Suite 710
Pasadena, California 91101
August 12, 2005

Mail Stop ASSIGNMENT RECORDATION SERVICES
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

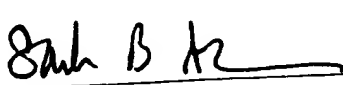
Sir:

We are transmitting herewith the attached:

- ☒ Transmittal sheet, in duplicate, containing Certificate under 37 CFR 1.8.
- ☒ Recordation Form Cover Sheet (1 page)
- ☒ Assignment (4 pages)
- ☒ A check in the amount of \$40.00 to cover the fee
- ☒ Return postcard

Please charge any additional fees or credit overpayment to Deposit Account No. 50-0306. A duplicate of this sheet is enclosed.

MANDEL & ADRIANO
55 S. Lake Avenue, Suite 710
Pasadena, California 91101
(626)395-7801

By: 
Name: Sarah B. Adriano
Reg. No.: 34,470
Initials: SBA



**RECORDATION FORM COVER SHEET
PATENTS ONLY**

To the Director of the U.S. Patent and Trademark Office: Please record the attached documents or the new address(es) below.

1. Name of conveying party(ies)

Robert J. Peach
Jurgen Bajorath

Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No

3. Nature of conveyance/Execution Date(s):

Execution Date(s) Jul 15, 2005; Aug 3, 2005

- ☒ Assignment ☐ Merger
☐ Security Agreement ☐ Change of Name
☐ Joint Research Agreement
☐ Government Interest Assignment
☐ Executive Order 9424, Confirmatory License
☐ Other _____

2. Name and address of receiving party(ies)

Name: Bristol-Myers Squibb Company

Internal Address: _____

Street Address: Lawrenceville-Princeton Road

City: Princeton

State: New Jersey

Country: US Zip: 08543

Additional name(s) & address(es) attached? ☐ Yes ☒ No

4. Application or patent number(s):

A. Patent Application No.(s)

☐ This document is being filed together with a new application.

B. Patent No.(s)

6,090,914

Additional numbers attached? ☐ Yes ☒ No

5. Name and address to whom correspondence concerning document should be mailed:

Name: Sarah B. Adriano

Internal Address: Mandel & Adriano

Street Address: 55 S. Lake Avenue, Suite 710

City: Pasadena

State: California Zip: 91101

Phone Number: 626.395.7801

Fax Number: 626.395.0694

Email Address: sbadriano@mandeladriano.com

6. Total number of applications and patents involved: 1

7. Total fee (37 CFR 1.21(h) & 3.41) \$ 40.00

- ☐ Authorized to be charged by credit card
☒ Authorized to be charged to deposit account
☒ Enclosed
☐ None required (government interest not affecting title)

8. Payment Information

a. Credit Card Last 4 Numbers _____
Expiration Date _____

b. Deposit Account Number 50-0306

Authorized User Name Sarah B. Adriano

9. Signature:

Sarah B. Adriano

Signature

August 12, 2005

Date

Sarah B. Adriano

Name of Person Signing

Total number of pages including cover sheet, attachments, and documents:

8

ASSIGNMENT

WHEREAS, I/WE, Robert J. Peach residing at 12848 Via Caballo Rojo, San Diego, California 92129, Jurgen Bajorath working at B-IT Intl. Center for Information Technology, Rheinische Friedrich-Wilhelms-University Bonn G6rresstra6e 13 D-53113 Bonn, Germany, made certain new and useful inventions and improvements for which I/WE filed an application for Letters Patent of the United States as application Serial No. 08/228,208 filed on April 15, 1994, Now U.S. Patent No. 6,090,914 issued on July 18, 2000 which is entitled CTLA4/CD28Ig HYBRID FUSION PROTEINS AND USES THEREOF;

AND WHEREAS, Bristol-Myers Squibb Company, a Delaware corporation, and having a place of business at Lawrenceville-Princeton Road, Princeton, New Jersey 08453-4000 (hereinafter "Assignee") is desirous of acquiring the entire right, title, and interest in and to said inventions, improvements and application, and in and to the Letters Patent to be obtained therefor;

NOW THEREFORE, to all whom it may concern, be it known that pursuant to contractual obligations heretofore assumed by ME/US and/or for other valuable and sufficient consideration, the receipt of which is hereby acknowledged, I/WE have sold, assigned, and transferred, and by these presents do sell, assign and transfer unto said Assignee, its successors or assigns, the entire right, title, and interest for all countries in and to all inventions and improvements disclosed in the aforesaid application, and in and to the application, all divisions, continuations, or renewals thereof, all Letters Patent which may be granted therefrom, and all reissues or extensions of such patents, and in and to any and all applications which have been or shall be filed in any foreign countries for Letters Patent on the inventions and improvements, including an assignment of all rights under the provisions of the International Convention, and all Letters Patent of foreign countries which may be granted therefrom; and I/WE do hereby authorize and request the Commissioner of Patents and Trademarks to issue any and all United States Letters Patent for the aforesaid inventions and improvements to the Assignee as the

assignee of the entire right, title and interest in and to the same, for the use of the Assignee, its successors and assigns.

AND, for the consideration aforesaid, I/WE do hereby agree that I/WE and MY/OUR executors and legal representatives will make, execute and deliver any and all other instruments in writing including any and all further application papers, affidavits, assignments and other documents, and will communicate to said Assignee, its successors and representatives all facts known to ME/US relating to said improvements and the history thereof and will testify in all legal proceedings and generally do all things which may be necessary or desirable more effectually to secure to and vest in said Assignee, its successors or assigns the entire right, title and interest in and to the improvements, inventions, applications, Letters Patent, rights, titles, benefits, privileges and advantages hereby sold, assigned and conveyed, or intended so to be.

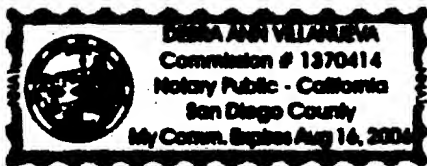
AND, furthermore I/WE covenant and agree with said Assignee, its successors and assigns, that no assignment, grant, mortgage, license or other agreement affecting the rights and priority herein conveyed has been made to others by ME/US and that full right to convey the same as herein expressed is possessed by ME/US.

IN TESTIMONY WHEREOF, I have hereunto set my hand this 15th day
of JULY, 2005.

Robert J. Peach
Robert J. Peach

STATE OF CALIFORNIA)
)
COUNTY OF SAN DIEGO)

On July 15, 2005 before me, DEBRA ANN VILLANUEVA personally
appeared ROBERT J. PEACH, ☒ personally
known to me OR ☐ proved to me on the basis of
satisfactory evidence to be the person whose name
is subscribed to the within instrument and
acknowledged to me that he executed the same in
his authorized capacity, and that by his signature on
the instrument the person, or the entity upon behalf
of which the person acted, executed the instrument.




[SEAL]

WITNESS my hand and official seal

Debra Ann Villanueva
Notary Public

ON0085D / M&A 30436.30US01

IN TESTIMONY WHEREOF, I have hereunto set my hand this four day
of August, 2005.



Jurgen Bajorath

BEST AVAILABLE COPY

FROM Merchant & Gould 310 44

(THU) 8.14.97 14 T.14:54/NO.4261403915 P.7

ASSIGNMENT

This Assignment as dated herein below, by Peter S. Linsley of 2430 9th Avenue West, Seattle, Washington 98119; Jeffrey A. Ledbetter, of 306 N.W. 113th Place, Seattle, Washington 98117; Nacin K. Dangle, of 11717 S.E. 60th Place, Renton, Washington; William Brady, of 618 219th Place, S.W., Bothell, Washington, 98021, and Philip M. Wallace of 3020 64 Avenue Southwest, Seattle, Washington 98116; Assignors, to BRISTOL-MYERS SQUIBB COMPANY, Assignee, a corporation, having a place of business at 3005 First Avenue, Seattle, Washington, 98121.

WHEREAS, Assignors have invented a new and useful METHOD FOR REGULATING THE IMMUNE RESPONSE USING CILIA4-BINDING MOLECULES AND IL4-BINDING MOLECULES, U.S. Serial No. 08/008,898, filed January 22, 1993; and

WHEREAS, Assignors believe themselves to be the original, joint inventors of the invention disclosed and claimed in said application for Letters Patent; and

WHEREAS, Assignee desires to acquire by formal, recordable assignment the entire right, title and interest in and to said invention, said application and any Letters Patent that may be granted for said invention in the United States and throughout the world;

NOW, THEREFORE, in consideration of the sum of One Dollar (\$1.00), and of other good and valuable consideration, the receipt and sufficiency of which are hereby acknowledged, Assignors hereby sell, assign, and transfer to Assignee, the entire right, title and interest in and to said invention, said application and any Letters Patent that may be granted for said invention in the United States and throughout the world, including the right

6567 0839

FROM Merchant & Gould 310 44

(THU) B. 14' 97 14 T. 14:54/NO. 4261403915 ?

to file foreign applications directly in the name of the Assignee and to claim for any such foreign applications any priority rights to which such applications are entitled under international conventions, treaties, or otherwise.

Further, Assignors agree that, upon request and without further compensation, but at no expense to Assignors, they and their legal representatives and assigns will do all lawful acts, including the execution of papers and the giving of testimony, that may be necessary or desirable for obtaining, sustaining, reissuing, or enforcing Letters Patent in the United States and throughout the world for said invention, and for perfecting, recording, or maintaining the title of Assignee, its successors and assigns, to said invention, said application, and any Letters Patent granted for said invention in the United States and throughout the world.

Assignors represent and warrant that they have not granted and will not grant to others any rights inconsistent with the rights granted herein.

Assignors authorize and request the Commissioner of Patents and Trademarks of the United States and of all foreign countries to issue any Letters Patent granted for said invention, whether on said application or on any subsequently filed division, continuation, continuation-in-part or reissue application, to Assignee, its successors and assigns, as the assignee of the entire interest in said invention.

5567

0840

OCT.11.2001 9:39AM

3 252 4526

NO.391 P.4

FROM Merchant & Gould 910 44

(THU) 8.14' 97 14

T.14:54/NO. A261403915 5 9

IN WITNESS WHEREOF, Assignor has executed this Assignment on
the dates as written.

Dated: 012293

ASSIGNOR

Peter S. Linsley
PETER S. LINSLEY

COUNTY OF Fry
STATE OF Washington

On this 22nd day of January, the year 1993, before
me personally appeared PETER S. LINSLEY personally known to me or
proved to me on the basis of satisfactory evidence to be the
person whose name is subscribed to this instrument, and
acknowledged to me that he executed it.

IN WITNESS WHEREOF, I have hereunto set my hand and affixed
my official seal the day and year in this certificate first above
written.

Lois Kay Bulen
NOTARY PUBLIC

1980 1993 6567 0811

OCT.11.2001 9:39AM

9 252 4526

NO.391 P.5

FROM Merchant & Gould 310 44

(THU) 8.14'97 16

T.14:54/NO.4261403915 P.10

IN WITNESS WHEREOF, Assignor has executed this
Assignment on the dates as written.

Dated: Jan 22, 1993

ASSIGNOR

Jeffrey A. Ledbetter
JEFFREY A. LEDBETTER

COUNTY OF King
STATE OF Washington

On this 22nd day of January, the year 1993, before me
personally appeared JEFFREY A. LEDBETTER personally known to me
or proved to me on the basis of satisfactory evidence to be the
person whose name is subscribed to this instrument; and
acknowledged to me that he executed it.

IN WITNESS WHEREOF, I have hereunto set my hand and affixed
my official seal the day and year in this certificate first above
written.

Ana Kay Butler
NOTARY PUBLIC

6567 0842

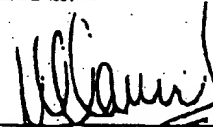
FROM Merchant & Gold 310 46

(THU) 8.14.97 14:54/NO.4261403915 P.11

IN WITNESS WHEREOF, Assignor has executed this
Assignment on the dates as written.

Dated: 1-28-93

ASSIGNOR


NITIN K. DAHLE


COUNTY OF

STATE OF

King
Washington

On this 28th day of January, the year 1993,
before me personally appeared NITIN K. DAHLE personally known to
me or proved to me on the basis of satisfactory evidence to be
the person whose name is subscribed to this instrument, and
acknowledged to me that he executed it.

IN WITNESS WHEREOF, I have hereunto set my hand and affixed
my official seal the day and year in this certificate first above
written.


NOTARY PUBLICREEL
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0843

OCT. 11. 2001 9:39AM

252 4526

NO. 391 P. 7

FROM Merchant & Gould 310 44

(THU) 8.14.97 14 T. 14:54/NO. 4261403915 P 12

IN WITNESS WHEREOF, Assignor has executed this Assignment on
the dates as written.
dated 01/22/93

ASSIGNOR

Will. Brady
WILLIAM BRADY

COUNTY OF King
STATE OF Washington

On this 22nd day of January, the year 1993, before
me personally appeared WILLIAM BRADY personally known to me or
proved to me on the basis of satisfactory evidence to be the
person whose name is subscribed to this instrument, and
acknowledged to me that he executed it.

IN WITNESS WHEREOF, I have hereunto set my hand and affixed
my official seal the day and year in this certificate first above
written.

Lois Kay Bulen
NOTARY PUBLIC

6567-1-956
4480

OCT.11.2001

9:39AM

9 252 4526

NO.391 P.8

FROM Merchant & Gould 310 4

(THU) 8.14'97 1. T.14:54/NO.4261403915 P 15

IN WITNESS WHEREOF, Assignor has executed this
Assignment on the dates as written.

Dated: 1/22/93

ASSIGNOR

Philip N. Wallace
PHILIP N. WALLACE

COUNTY OF Puy
STATE OF Washington

On this 22nd day of January, the year 1993,
before me personally appeared PHILIP N. WALLACE personally known
to me or proved to me on the basis of satisfactory evidence to be
the person whose name is subscribed to this instrument, and
acknowledged to me that he executed it.

IN WITNESS WHEREOF, I have hereunto set my hand and affixed
my official seal the day and year in this certificate first above
written.

Lori Kay Bulen
NOTARY PUBLIC

RECORDED
PATENT AND TRADEMARK
OFFICE

APR 19 1993

1 Dec 94

PATENT
7848ASSIGNMENT

This Assignment as dated herein below, by Peter S. Linsley of 2430 9th Avenue West, Seattle, Washington 98119; Jeffrey A. Ledbetter, of 306 N.W. 113th Place, Seattle, Washington 98117; Nitin K. Damle, of 11717 S.E. 60th Place, Renton, Washington; and William Brady, of 618 219th Place, S.W., Bothell, Washington, 98021; Assignors, to BRISTOL-MYERS SQUIBB COMPANY, Assignee, a corporation, having a place of business at 3005 First Avenue, Seattle, Washington, 98121.

WHEREAS, Assignors have invented a new and useful CTLA4 RECEPTOR AND METHODS FOR ITS USE, for which an application for United States Letters Patent has been filed on June 27, 1991, under Serial Number 07/723,617; and

WHEREAS, Assignors believe themselves to be the original, joint inventors of the invention disclosed and claimed in said application for Letters Patent; and

WHEREAS, Assignee desires to acquire by formal, recordable assignment the entire right, title and interest in and to said invention, said application and any Letters Patent that may be granted for said invention in the United States and throughout the world;

NOW, THEREFORE, in consideration of the sum of One Dollar (\$1.00) and of other good and valuable consideration, the receipt and sufficiency of which are hereby acknowledged, Assignors hereby sell, assign, and transfer to Assignee, the entire right, title, and interest in and to said invention, said application and any Letters Patent that may be granted for said invention in the United States and throughout the world, including the right to file foreign applications

REU5821 FRANK588

BEST AVAILABLE COPY

PATENT
7848

directly in the name of the Assignee and to claim for any such foreign applications any priority rights to which such applications are entitled under international conventions, treaties, or otherwise.

Further, Assignors agree that, upon request and without further compensation, but at no expense to Assignor, they and their legal representatives and assigns will do all lawful acts, including the execution of papers and the giving of testimony, that may be necessary or desirable for obtaining, sustaining, reissuing, or enforcing Letters Patent in the United States and throughout the world for said invention, and for perfecting, recording, or maintaining the title of Assignee, its successors and assigns, to said invention, said application, and any Letters Patent granted for said invention in the United States and throughout the world.

Assignors represent and warrant that they have not granted and will not grant to others any rights inconsistent with the rights granted herein.

Assignors authorize and request the Commissioner of Patents and Trademarks of the United States and of all foreign countries to issue any Letters Patent granted for said invention, whether on said application or on any subsequently filed division, continuation, continuation-in-part or reissue application, to Assignee, its successors and assigns, as the assignee of the entire interest in said invention.

BEST AVAILABLE COPY

REEL 5821 FRAME 589

PATENT
7848

IN WITNESS WHEREOF, Assignor has executed this Assignment
on the dates as written.

Dated: 081491

ASSIGNOR

Peter S. Linsley
PETER S. LINSLEY

COUNTY OF
STATE OF

King
Washington

On this 14 day of August, the year 91,
before me personally appeared PETER S. LINSLEY personally
known to me or proved to me on the basis of satisfactory
evidence to be the person whose name is subscribed to this
instrument, and acknowledged to me that he executed it.

IN WITNESS WHEREOF, I have hereunto set my hand and
affixed my official seal the day and year in this certificate
first above written.

Tawny K. Randall
NOTARY PUBLIC

TAWNY K RANDALL
Notary Public
STATE OF WASHINGTON
My Commission Expires
August 25, 1993

REC-5821 FRANK590

BEST AVAILABLE COPY

PATENT
7848

IN WITNESS WHEREOF, Assignor has executed this Assignment
on the dates as written.

Dated: 9/8/91

ASSIGNOR

Jeffrey A. Ledbetter
JEFFREY A. LEDBETTER

COUNTY OF

STATE OF

King
Washington.

On this 8 day of August, the year 1991,
before me personally appeared JEFFREY A. LEDBETTER personally
known to me or proved to me on the basis of satisfactory
evidence to be the person whose name is subscribed to this
instrument, and acknowledged to me that he executed it.

IN WITNESS WHEREOF, I have hereunto set my hand and
affixed my official seal the day and year in this certificate
first above written.

Tawny K. Randall
NOTARY PUBLIC

TAWNY K RANDALL
Notary Public
STATE OF WASHINGTON
My Commission Expires
August 25, 1993

BEST AVAILABLE COPY

REEL 5821 FRAME 591

PATENT
7848

IN WITNESS WHEREOF, Assignor has executed this Assignment
on the dates as written.

Dated: 8/8/91

ASSIGNOR


NITIN K. DAMLE

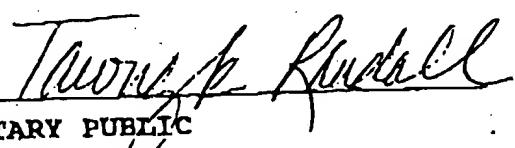
COUNTY OF

STATE OF

King
Washington

On this 8 day of August, the year 1991,
before me personally appeared NITIN K. DAMLE personally known
to me or proved to me on the basis of satisfactory evidence to
be the person whose name is subscribed to this instrument, and
acknowledged to me that he executed it.

IN WITNESS WHEREOF, I have hereunto set my hand and
affixed my official seal the day and year in this certificate
first above written.


NOTARY PUBLIC

TAWNY K RANDALL
Notary Public
STATE OF WASHINGTON
My Commission Expires
August 25, 1993

BEST AVAILABLE COPY

REEL 5821 FRAME 592

PATENT
7848

IN WITNESS WHEREOF, Assignor has executed this Assignment
on the dates as written.

Dated: 8/8/91

ASSIGNOR

William Brady
WILLIAM BRADY

COUNTY OF
STATE OF

King
Washington

On this 8 day of August, the year 91,
before me personally appeared WILLIAM BRADY personally known
to me or proved to me on the basis of satisfactory evidence to
be the person whose name is subscribed to this instrument, and
acknowledged to me that he executed it.

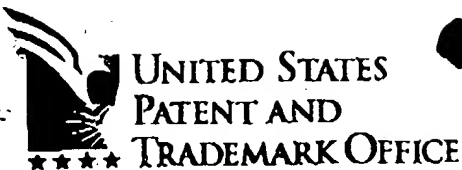
IN WITNESS WHEREOF, I have hereunto set my hand and
affixed my official seal the day and year in this certificate
first above written.

Tawny K. Randall
NOTARY PUBLIC

TAWNY K RANDALL
Notary Public
STATE OF WASHINGTON
My Commission Expires
August 26, 1993

BEST AVAILABLE COPY

REC-5821-1A-1593



MARCH 11, 2002

PTAS

Chief Information Officer
Washington, DC 20231
www.uspto.gov

AUDREY F. SHER, ESQ.
ROUTE 206 AND PROVINCE LINE ROAD
PRINCETON, NEW JERSEY 08540



101950737A

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 01/04/2002

REEL/FRAME: 012460/0666
NUMBER OF PAGES: 8

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
LINSLEY, PETER S.

DOC DATE: 10/16/2001

ASSIGNOR:
BRADY, WILLIAM

DOC DATE: 11/01/2001

ASSIGNEE:
BRISTOL-MYERS SQUIBB COMPANY
LAWRENCEVILLE-PRINCETON ROAD
PRINCETON, NEW JERSEY 08543-4000

SERIAL NUMBER: 09609915
PATENT NUMBER:

FILING DATE: 07/03/2000
ISSUE DATE:

RECEIVED
BMS PATENT LAW

SHARON LATIMER, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

MAR 19 2002

Docketed Item _____
Due Date _____
Attorney H. Sher

BEST AVAILABLE COPY

FORM PTO-1619A
Expires 08/30/98
OMB 0651-0027

JAN - 4 2002

01-17-2002



101950737

1-4-02

**RECORDATION FORM COVER SHEET
PATENTS ONLY**

TO: The Commissioner of Patents and Trademarks: Please record the attached original document(s) or copy(ies).

Submission Type

- ☒ New
- ☐ Resubmission (Non-Recordation)
Document ID#
- ☐ Correction of PTO Error
Reel # Frame #
- ☐ Corrective Document
Reel # Frame #

Conveyance Type

- ☒ Assignment ☐ Security Agreement
- ☐ License ☐ Change of Name
- ☐ Merger ☐ Other
- U.S. Government**
(For Use ONLY by U.S. Government Agencies)
- ☐ Departmental File ☐ Secret File

Conveying Party(ies)

☐ Mark if additional names of conveying parties attached

Name (line 1)

Execution Date
Month Day Year

Name (line 2)

Execution Date
Month Day Year

Second Party

Name (line 1)

Name (line 2)

Receiving Party

☐ Mark if additional names of receiving parties attached

Name (line 1)

☐ If document to be recorded is an assignment and the receiving party is not domiciled in the United States, an appointment of a domestic representative is attached. (Designation must be a separate document from Assignment.)

Name (line 2)

Address (line 1)

Address (line 2)

Address (line 3)

City

State/Country

Zip Code

Domestic Representative Name and Address

Enter for the first Receiving Party only.

Name

Address (line 1)

Address (line 2)

Address (line 3)

Address (line 4)

FOR OFFICE USE ONLY

01/17/2002 JILLIAN2 00000003 09609915

01 FC:381

80.00 DP

Public burden reporting for this collection of information is estimated to average approximately 30 minutes per Cover Sheet to be recorded, including time for reviewing the document and gathering the data needed to complete the Cover Sheet. Send comments regarding this burden estimate to the U.S. Patent and Trademark Office, Chief Information Officer, Washington, D.C. 20231 and to the Office of Information and Regulatory Affairs, Office of Management and Budget, Paperwork Reduction Project (0651-0027), Washington, D.C. 20503. See OMB Information Collection Budget Package 0651-0027, Patent and Trademark Assignment Practice. DO NOT SEND REQUESTS TO RECORD ASSIGNMENT DOCUMENTS TO THIS ADDRESS.

Mail documents to be recorded with required cover sheet(s) information to:
Commissioner of Patents and Trademarks, Box Assignments, Washington, D.C. 20231

Correspondent Name and Address

Area Code and Telephone Number (609)252 3218

Name Audrey F. Sher, Esq.

Address (line 1) Route 206 and Province Line Road

Address (line 2) Princeton, New Jersey 08540

Address (line 3)

Address (line 4)

Pages

Enter the total number of pages of the attached conveyance document including any attachments.

6

Application Number(s) or Patent Number(s)

☐ Mark if additional numbers attached

Enter either the Patent Application Number or the Patent Number (DO NOT ENTER BOTH numbers for the same property).

Patent Application Number(s)

09/609,915

Patent Number(s)

If this document is being filed together with a new Patent Application, enter the date the patent application was signed by the first named executing inventor.

Month Day Year

Patent Cooperation Treaty (PCT)

Enter PCT application number only if a U.S. Application Number has not been assigned.

PCT PCT PCT
PCT PCT PCT

Number of Properties

Enter the total number of properties involved.

2

Fee Amount

Fee Amount for Properties Listed (37 CFR 3.41): \$ 80.00

Method of Payment:

Deposit Account

(Enter for payment by deposit account or if additional fees can be charged to the account.)

Enclosed ☐ Deposit Account ☐

Deposit Account Number:

50-0306

Authorization to charge additional fees:

Yes ☒ No ☐

Statement and Signature

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document. Charges to deposit account are authorized, as indicated herein.

Sarah B. Adriano

Name of Person Signing

Sarah B. Adriano

Signature

November 5, 2001

Date

ASSIGNMENT

WHEREAS, I/WE, Peter S. Linsley residing at 2430 9th Avenue West, Seattle, Washington 98119, Jeffrey A. Ledbetter residing at 306 N. W. 113th Place, Seattle, Washington 98117, Jurgen Bajorath residing at 17406 37th Avenue W, Lynnwood, Washington 98037, Robert James Peach residing at 12848 Via Caballo Rojo, San Diego, California 92129, William Brady residing at 618 219th Place SW, Bothell, Washington 98021, Philip Wallace residing at 3020 64th Avenue Southwest #D, Seattle, Washington 98116, Nitin Damle residing at 53 Stevenson Lane, Upper Saddle River, New Jersey 07458, made certain new and useful inventions and improvements for which I/WE filed an application for Letters Patent of the United States as application Serial No. 09/609,915 filed on July 3, 2000 which is entitled SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF;

AND WHEREAS, Bristol-Myers Squibb Company, a Delaware corporation, and having a place of business at Lawrenceville-Princeton Road, Princeton, New Jersey 08453-4000 (hereinafter "Assignee") is desirous of acquiring the entire right, title, and interest in and to said inventions, improvements and application, and in and to the Letters Patent to be obtained therefor;

NOW THEREFORE, to all whom it may concern, be it known that pursuant to contractual obligations heretofore assumed by ME/US and/or for other valuable and sufficient consideration, the receipt of which is hereby acknowledged, I/WE have sold, assigned, and transferred, and by these presents do sell, assign and transfer unto said Assignee, its successors or assigns, the entire right, title, and interest for all countries in and to all inventions and improvements disclosed in the aforesaid application, and in and to the application, all divisions, continuations, or renewals thereof, all Letters Patent which may be granted therefrom, and all reissues or extensions of such patents, and in and to any and all applications which have been or shall be filed in any foreign countries for Letters Patent on the inventions and improvements, including an assignment of all rights under the provisions of the International Convention, and all Letters Patent of

foreign countries which may be granted therefrom; and I/WE do hereby authorize and request the Commissioner of Patents and Trademarks to issue any and all United States Letters Patent for the aforesaid inventions and improvements to the Assignee as the assignee of the entire right, title and interest in and to the same, for the use of the Assignee, its successors and assigns.

AND, for the consideration aforesaid, I/WE do hereby agree that I/WE and MY/OUR executors and legal representatives will make, execute and deliver any and all other instruments in writing including any and all further application papers, affidavits, assignments and other documents, and will communicate to said Assignee, its successors and representatives all facts known to ME/US relating to said improvements and the history thereof and will testify in all legal proceedings and generally do all things which may be necessary or desirable more effectually to secure to and vest in said Assignee, its successors or assigns the entire right, title and interest in and to the improvements, inventions, applications, Letters Patent, rights, titles, benefits, privileges and advantages hereby sold, assigned and conveyed, or intended so to be.

AND, furthermore I/WE covenant and agree with said Assignee, its successors and assigns, that no assignment, grant, mortgage, license or other agreement affecting the rights and priority herein conveyed has been made to others by ME/US and that full right to convey the same as herein expressed is possessed by ME/US.

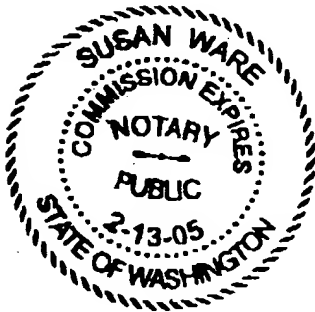
IN TESTIMONY WHEREOF, I have hereunto set my hand this 1st day
of NOVEMBER, 2001.

William Brady
William Brady

STATE OF Washington
COUNTY OF King

On 11/01, before me, _____ personally
appeared William Brady, ☐ personally
known to me OR ☒ proved to me on the basis of
satisfactory evidence to be the person whose name
is subscribed to the within instrument and
acknowledged to me that he executed the same in
his authorized capacity, and that by his signature on
the instrument the person, or the entity upon behalf
of which the person acted, executed the instrument.

WITNESS my hand and official seal



[SEAL]

Susan Ware
Notary Public

ASSIGNMENT

WHEREAS, I/WE, Peter S. Linsley residing at 2430 9th Avenue West, Seattle, Washington 98119, Jeffrey A. Ledbetter residing at 306 N. W. 113th Place, Seattle, Washington 98117, Jurgen Bajorath residing at 17406 37th Avenue W, Lynnwood, Washington 98037, Robert James Peach residing at 12848 Via Caballo Rojo, San Diego, California 92129, William Brady residing at 618 219th Place SW, Bothell, Washington 98021, Philip Wallace residing at 3020 64th Avenue Southwest #D, Seattle, Washington 98116, Nitin Damle residing at 53 Stevenson Lane, Upper Saddle River, New Jersey 07458, made certain new and useful inventions and improvements for which I/WE filed an application for Letters Patent of the United States as application Serial No. 09/609,915 filed on July 3, 2000 which is entitled SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF;

AND WHEREAS, Bristol-Myers Squibb Company, a Delaware corporation, and having a place of business at Lawrenceville-Princeton Road, Princeton, New Jersey 08453-4000 (hereinafter "Assignee") is desirous of acquiring the entire right, title, and interest in and to said inventions, improvements and application, and in and to the Letters Patent to be obtained therefor;

NOW THEREFORE, to all whom it may concern, be it known that pursuant to contractual obligations heretofore assumed by ME/US and/or for other valuable and sufficient consideration, the receipt of which is hereby acknowledged, I/WE have sold, assigned, and transferred, and by these presents do sell, assign and transfer unto said Assignee, its successors or assigns, the entire right, title, and interest for all countries in and to all inventions and improvements disclosed in the aforesaid application, and in and to the application, all divisions, continuations, or renewals thereof, all Letters Patent which may be granted therefrom, and all reissues or extensions of such patents, and in and to any and all applications which have been or shall be filed in any foreign countries for Letters Patent on the inventions and improvements, including an assignment of all rights under the provisions of the International Convention, and all Letters Patent of

foreign countries which may be granted therefrom; and I/WE do hereby authorize and request the Commissioner of Patents and Trademarks to issue any and all United States Letters Patent for the aforesaid inventions and improvements to the Assignee as the assignee of the entire right, title and interest in and to the same, for the use of the Assignee, its successors and assigns.

AND, for the consideration aforesaid, I/WE do hereby agree that I/WE and MY/OUR executors and legal representatives will make, execute and deliver any and all other instruments in writing including any and all further application papers, affidavits, assignments and other documents, and will communicate to said Assignee, its successors and representatives all facts known to ME/US relating to said improvements and the history thereof and will testify in all legal proceedings and generally do all things which may be necessary or desirable more effectually to secure to and vest in said Assignee, its successors or assigns the entire right, title and interest in and to the improvements, inventions, applications, Letters Patent, rights, titles, benefits, privileges and advantages hereby sold, assigned and conveyed, or intended so to be.

AND, furthermore I/WE covenant and agree with said Assignee, its successors and assigns, that no assignment, grant, mortgage, license or other agreement affecting the rights and priority herein conveyed has been made to others by ME/US and that full right to convey the same as herein expressed is possessed by ME/US.

IN TESTIMONY WHEREOF, I have hereunto set my hand this 16 day
of Oct, 20 01.

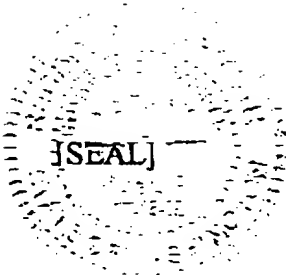
Peter S. Linsley
Peter S. Linsley

STATE OF WASHINGTON)

COUNTY OF KING)

On 10.16.01, before me, REGINA WARMUTH personally
appeared PETER S. LINSLEY, ☒ personally
known to me OR ☐ proved to me on the basis of
satisfactory evidence to be the person whose name
is subscribed to the within instrument and
acknowledged to me that he executed the same in
his authorized capacity, and that by his signature on
the instrument the person, or the entity upon behalf
of which the person acted, executed the instrument.

WITNESS my hand and official seal



Regina Warmuth

Notary Public

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Peter S. Linsley et al.
Serial No.: 09/609,915
Filed: July 3, 2000
Docket: 30436.30US12
Title: SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

CERTIFICATE UNDER 37 CFR 1.8

I hereby certify that this paper or fee is being deposited with the United States Postal as first class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 on November 5, 2001.

By: _____

Name: Richelle Ann Dominguez

35 N. Arroyo Parkway, Suite 60
Pasadena, California 91103
November 5, 2001

BOX ASSIGNMENTS

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

We are transmitting herewith the attached:

- ☒ Transmittal sheet, in duplicate, containing Certificate under 37 CFR 1.8.
- ☒ Recordation Form Cover Sheet (PTO-1619A,B)
- ☒ Executed Assignments (2 sets)
- ☒ A check in the amount of \$80.00
- ☒ Return postcard

Please charge any additional fees or credit overpayment to Deposit Account No. 50-0306. A duplicate of this sheet is enclosed.

MANDEL & ADRIANO

35 No. Arroyo Parkway, Suite 60
Pasadena, California 91103
(626)395-7801

By: _____

Sarah B. Adriano
Name: Sarah B. Adriano
Reg. No.: 34,470
Initials: SBA
Customer No.: 26941

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Peter S. Linsley et al.
Serial No.: 09/609,915
Filed: July 3, 2000
Docket: 30436 30USI2
Title: SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

CERTIFICATE UNDER 37 CFR 1.8

I hereby certify that this paper or fee is being deposited with the United States Postal as first class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 on November 5, 2001.

By: 
Name: Richelle Ann Domingo

35 N. Arroyo Parkway, Suite 60
Pasadena, California 91103
November 5, 2001

BOX ASSIGNMENTS

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

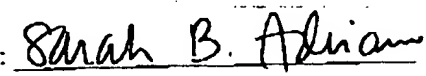
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Customer No.: 26941

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Peter S. Linsley et al.
Serial No.: 09/609,915
Filed: July 3, 2000
Docket: 30436.30USI2
Title: SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

CERTIFICATE UNDER 37 CFR 1.8

I hereby certify that this paper or fee is being deposited with the United States Postal Service as first class mail in an envelope addressed to the Mail Stop: Assignment Recordation Services, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313 on August 12, 2005.

By: 
Name: Richelle Ann Domingo

55 S. Lake Avenue, Suite 710
Pasadena, California 91101
August 12, 2005

Mail Stop ASSIGNMENT RECORDATION SERVICES
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

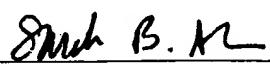
Sir:

We are transmitting herewith the attached:

- ☒ Transmittal sheet, in duplicate, containing Certificate under 37 CFR 1.8.
- ☒ Recordation Form Cover Sheet (1 page)
- ☒ Assignment (7 pages)
- ☒ A check in the amount of \$40.00 to cover the fee
- ☒ Return postcard

Please charge any additional fees or credit overpayment to Deposit Account No. 50-0306. A duplicate of this sheet is enclosed.

MANDEL & ADRIANO
55 S. Lake Avenue, Suite 710
Pasadena, California 91101
(626)395-7801

By: 
Name: Sarah B. Adriano
Reg. No.: 34,470
Initials: SBA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Peter S. Linsley et al.
Serial No.: 09/609,915
Filed: July 3, 2000
Docket: 30436.30USI2
Title: SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

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Name: Richelle Ann Domingo

55 S. Lake Avenue, Suite 710
Pasadena, California 91101
August 12, 2005

Mail Stop ASSIGNMENT RECORDATION SERVICES
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

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Please charge any additional fees or credit overpayment to Deposit Account No. 50-0306. A duplicate of this sheet is enclosed.

MANDEL & ADRIANO
55 S. Lake Avenue, Suite 710
Pasadena, California 91101
(626)395-7801

By: _____

Name: Sarah B. Adriano
Reg. No.: 34,470
Initials: SBA

**RECORDATION FORM COVER SHEET
PATENTS ONLY**

To the Director of the U.S. Patent and Trademark Office: Please record the attached documents or the new address(es) below.

1. Name of conveying party(ies)

Nitin Damle
Robert J. Peach
Jurgen Bajorath

Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No

3. Nature of conveyance/Execution Date(s):

Execution Date(s) Apr 25, 2005; Jul 15, 2005; Jul 26, 2005

- ☒ Assignment ☐ Merger
☐ Security Agreement ☐ Change of Name
☐ Joint Research Agreement
☐ Government Interest Assignment
☐ Executive Order 9424, Confirmatory License
☐ Other _____

2. Name and address of receiving party(ies)

Name: Bristol-Myers Squibb Company

Internal Address: _____

Street Address: Lawrenceville-Princeton Road

City: Princeton

State: New Jersey

Country: US Zip: 08543

Additional name(s) & address(es) attached? ☐ Yes ☒ No

4. Application or patent number(s):

☐ This document is being filed together with a new application.

A. Patent Application No.(s)
09/609,915

B. Patent No.(s)

Additional numbers attached? ☐ Yes ☒ No

5. Name and address to whom correspondence concerning document should be mailed:

Name: Sarah B. Adriano

Internal Address: Mandel & Adriano

Street Address: 55 S. Lake Avenue, Suite 710

City: Pasadena

State: California Zip: 91101

Phone Number: 626.395.7801

Fax Number: 626.395.0694

Email Address: sbadriano@mandeladriano.com

6. Total number of applications and patents involved: 1

7. Total fee (37 CFR 1.21(h) & 3.41) \$ 40.00

- ☐ Authorized to be charged by credit card
☒ Authorized to be charged to deposit account
☒ Enclosed
☐ None required (government interest not affecting title)

8. Payment Information

a. Credit Card Last 4 Numbers _____
Expiration Date _____

b. Deposit Account Number 50-0306

Authorized User Name Sarah B. Adriano

9. Signature:

Sarah B. Adriano

Signature

August 12, 2005

Date

Sarah B. Adriano
Name of Person Signing

Total number of pages including cover sheet, attachments, and documents:

11

ASSIGNMENT

WHEREAS, I/WE, Peter S. Linsley residing at 2430 9th Avenue West, Seattle, Washington 98119, Jeffrey A. Ledbetter residing at 306 N. W. 113th Place, Seattle, Washington 98117, Jurgen Bajorath residing at 17406 37th Avenue W, Lynnwood, Washington 98037, Robert James Peach residing at 12848 Via Caballo Rojo, San Diego, California 92129, William Brady residing at 618 219th Place SW, Bothell, Washington 98021, Philip Wallace residing at 3020 64th Avenue Southwest #D, Seattle, Washington 98116, Nitin Damle residing at 53 Stevenson Lane, Upper Saddle River, New Jersey 07458, made certain new and useful inventions and improvements for which I/WE filed an application for Letters Patent of the United States as application Serial No. 09/609,915 filed on July 3, 2000 which is entitled SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF;

AND WHEREAS, Bristol-Myers Squibb Company, a Delaware corporation, and having a place of business at Lawrenceville-Princeton Road, Princeton, New Jersey 08453-4000 (hereinafter "Assignee") is desirous of acquiring the entire right, title, and interest in and to said inventions, improvements and application, and in and to the Letters Patent to be obtained therefor;

NOW THEREFORE, to all whom it may concern, be it known that pursuant to contractual obligations heretofore assumed by ME/US and/or for other valuable and sufficient consideration, the receipt of which is hereby acknowledged, I/WE have sold, assigned, and transferred, and by these presents do sell, assign and transfer unto said Assignee, its successors or assigns, the entire right, title, and interest for all countries in and to all inventions and improvements disclosed in the aforesaid application, and in and to the application, all divisions, continuations, or renewals thereof, all Letters Patent which may be granted therefrom, and all reissues or extensions of such patents, and in and to any and all applications which have been or shall be filed in any foreign countries for Letters Patent on the inventions and improvements, including an assignment of all rights under the provisions of the International Convention, and all Letters Patent of

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AND, for the consideration aforesaid, I/WE do hereby agree that I/WE and MY/OUR executors and legal representatives will make, execute and deliver any and all other instruments in writing including any and all further application papers, affidavits, assignments and other documents, and will communicate to said Assignee, its successors and representatives all facts known to ME/US relating to said improvements and the history thereof and will testify in all legal proceedings and generally do all things which may be necessary or desirable more effectually to secure to and vest in said Assignee, its successors or assigns the entire right, title and interest in and to the improvements, inventions, applications, Letters Patent, rights, titles, benefits, privileges and advantages hereby sold, assigned and conveyed, or intended so to be.

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IN TESTIMONY WHEREOF, I have hereunto set my hand this 25th day
of April, 2005.

Nitin Damle

STATE OF New York
COUNTY OF Rockland

On 4/25/05, before me, Margie Abrams personally
appeared Nitin Damle, ☒ personally
known to me OR ☐ proved to me on the basis of
satisfactory evidence to be the person whose name
is subscribed to the within instrument and
acknowledged to me that he executed the same in
his authorized capacity, and that by his signature on
the instrument the person, or the entity upon behalf
of which the person acted, executed the instrument.

WITNESS my hand and official seal

[SEAL]

Margie E. Abrams
Notary Public

MARGIE E. ABRAMS
NOTARY PUBLIC, STATE OF NEW YORK
NO. 405013
QUALIFIED IN ROCKLAND COUNTY
COMMISSION EXPIRES 1/19/2007

ASSIGNMENT

WHEREAS, I/WE, Robert J. Peach residing at 12848 Via Caballo Rojo, San Diego, California 92129, Jurgen Bajorath working at B-IT Intl. Center for Information Technology, Rheinische Friedrich-Wilhelms-University Bonn Görresstraße 13 D-53113 Bonn, Germany, made certain new and useful inventions and improvements for which I/WE filed an application for Letters Patent of the United States as application Serial No. 09/609,915 filed on July 3, 2000 which is entitled SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF;

AND WHEREAS, Bristol-Myers Squibb Company, a Delaware corporation, and having a place of business at Lawrenceville-Princeton Road, Princeton, New Jersey 08453-4000 (hereinafter "Assignee") is desirous of acquiring the entire right, title, and interest in and to said inventions, improvements and application, and in and to the Letters Patent to be obtained therefor;

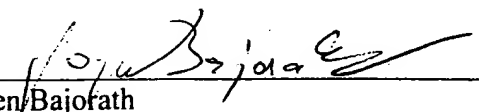
NOW THEREFORE, to all whom it may concern, be it known that pursuant to contractual obligations heretofore assumed by ME/US and/or for other valuable and sufficient consideration, the receipt of which is hereby acknowledged, I/WE have sold, assigned, and transferred, and by these presents do sell, assign and transfer unto said Assignee, its successors or assigns, the entire right, title, and interest for all countries in and to all inventions and improvements disclosed in the aforesaid application, and in and to the application, all divisions, continuations, or renewals thereof, all Letters Patent which may be granted therefrom, and all reissues or extensions of such patents, and in and to any and all applications which have been or shall be filed in any foreign countries for Letters Patent on the inventions and improvements, including an assignment of all rights under the provisions of the International Convention, and all Letters Patent of foreign countries which may be granted therefrom; and I/WE do hereby authorize and request the Commissioner of Patents and Trademarks to issue any and all United States Letters Patent for the aforesaid inventions and improvements to the Assignee as the

assignee of the entire right, title and interest in and to the same, for the use of the Assignee, its successors and assigns.

AND, for the consideration aforesaid, I/WE do hereby agree that I/WE and MY/OUR executors and legal representatives will make, execute and deliver any and all other instruments in writing including any and all further application papers, affidavits, assignments and other documents, and will communicate to said Assignee, its successors and representatives all facts known to ME/US relating to said improvements and the history thereof and will testify in all legal proceedings and generally do all things which may be necessary or desirable more effectually to secure to and vest in said Assignee, its successors or assigns the entire right, title and interest in and to the improvements, inventions, applications, Letters Patent, rights, titles, benefits, privileges and advantages hereby sold, assigned and conveyed, or intended so to be.

AND, furthermore I/WE covenant and agree with said Assignee, its successors and assigns, that no assignment, grant, mortgage, license or other agreement affecting the rights and priority herein conveyed has been made to others by ME/US and that full right to convey the same as herein expressed is possessed by ME/US.

IN TESTIMONY WHEREOF, I have hereunto set my hand this 26th day
of July, 2005.



Jurgen Bajofath

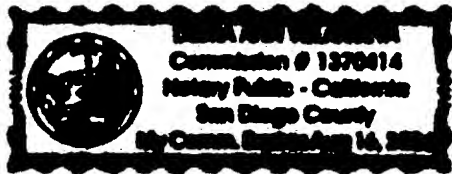
IN TESTIMONY WHEREOF, I have hereunto set my hand this 15th day
of JULY, 2005.

Robert J. Peach
Robert J. Peach

STATE OF CALIFORNIA)
)
COUNTY OF SAN DIEGO)

On July 15, 2005 before me, DEBRA ANN VILLANUEVA personally
appeared ROBERT J. PEACH, ☒ personally
known to me OR ☐ proved to me on the basis of
satisfactory evidence to be the person whose name
is subscribed to the within instrument and
acknowledged to me that he executed the same in
his authorized capacity, and that by his signature on
the instrument the person, or the entity upon behalf
of which the person acted, executed the instrument.

WITNESS my hand and official seal



[SEAL]

Debra Ann Villanueva
Notary Public

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley, Jeffrey Ledbetter, Jorgen Bajorath, Robert Peach,
William Brady, Philip Wallace, and Nitin Damle

Serial No. : 09/609,915 Examiner: Lorraine Spector, Ph.D.

Filed : July 3, 2000 Group Art Unit: 1647

For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

SIR/MADAM:

**DECLARATION BY PHILIP WALLACE
UNDER 37 C.F.R. §1.48(a)(2)**

I, Philip Wallace, submit this request to correct inventorship for the subject application under 37 C.F.R. §1.48(a) in view of the originally filed claims 1-25.

The inventive entity is set forth in error in the executed declaration under 37 C.F.R. §1.63 of the subject nonprovisional application. This error was inadvertent and arose without any deceptive intent on my part.

Upon reconsideration and further review, I, Philip Wallace am not an inventor of the originally filed claims 1-25, or claims 1-2, 5, 9 and 11-30 which are presently pending, and thus ask to be removed as an inventor of the claims of the subject application. This inadvertent error arose without any deceptive intent on my part.

Date: 9th March 2005 By: P. Wallace

Philip Wallace, Ph.D. D. Phil
PMW

United States Patent Application

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

The specification of which

- a. ☐ is attached hereto
 b. ☒ was filed on July 3, 2000 as application serial no. 09/609,915 and was amended on April 15, 2002, May 8, 2002 (hand delivered May 9, 2002), September 9, 2002, November 4, 2002, June 2, 2004 and August 2, 2004 (resubmitted on August 23, 2004) (if applicable) (in the case of a PCT-filed application) described and claimed in international no. filed and as amended on , which I have reviewed and for which I solicit a United States patent.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56 (attached hereto).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on the basis of which priority is claimed:

- a. ☐ no such applications have been filed.
 b. ☐ such applications have been filed as follows:

FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC § 119			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)
ALL FOREIGN APPLICATION(S), IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S)			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)

I hereby claim the benefit under Title 35, United States Code, § 120/365 of any United States and PCT international application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. APPLICATION NUMBER	DATE OF FILING (day, month, year)	STATUS (patented, pending, abandoned)
07/723,617	27 June 1991	Abandoned
08/008,898	22 January 1993	Patented
08/228,208	15 April 1994	Patented

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

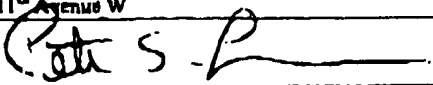
U.S. PROVISIONAL APPLICATION NUMBER	DATE OF FILING (Day, Month, Year)

I hereby appoint the following attorneys and agents associated with Customer No. 23914, respectively and individually, as my attorneys and agents, with full power of substitution, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Please direct all communication to the address associated with Customer No. 23914, which is currently:

Stephen B. Davis
Bristol-Myers Squibb Company
Patent Department
P.O. Box 4000
Princeton, New Jersey 08543-4000

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2	Full Name Of Inventor	Family Name Linsley	First Given Name Peter	Second Given Name S.
0	Residence & Citizenship	City Seattle	State or Foreign Country Washington	Country of Citizenship USA
1	Post Office Address	Post Office Address 2528 11 th Avenue W	City Seattle	State & Zip Code/Country Washington, 98119/USA
Signature of Inventor 201: 				Date: 01/03/05 ^{US} _{PG} 112805
2	Full Name Of Inventor	Family Name Lefebvre	First Given Name Jeffrey	Second Given Name A.
0	Residence & Citizenship	City Seattle	State or Foreign Country Washington	Country of Citizenship USA
2	Post Office Address	Post Office Address 306 N.W. 113 th Place	City Seattle	State & Zip Code/Country Washington, 98117/USA
Signature of Inventor 202: _____				Date: _____
2	Full Name Of Inventor	Family Name Bajorath	First Given Name Jurgen	Second Given Name
0	Residence & Citizenship	City Lynnwood	State or Foreign Country Washington	Country of Citizenship Germany
3	Post Office Address	Post Office Address 17406 37 th Avenue W.	City Lynnwood	State & Zip Code/Country Washington, 98037/USA
Signature of Inventor 203: _____				Date: _____

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

U.S. PROVISIONAL APPLICATION NUMBER	DATE OF FILING (Day, Month, Year)

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201	Full Name Of Inventor	Family Name Linsley	First Given Name Peter	Second Given Name S.
	Residence & Citizenship	City Seattle	State or Foreign Country Washington	Country of Citizenship USA
	Post Office Address	Post Office Address 2528 11 th Avenue W.	City Seattle	State & Zip Code/Country Washington, 98119/USA
Signature of Inventor 201:			Date:	
202	Full Name Of Inventor	Family Name Ledbetter	First Given Name Jeffrey	Second Given Name A.
	Residence & Citizenship	City Seattle	State or Foreign Country Washington	Country of Citizenship USA
	Post Office Address	Post Office Address 306 N.W. 113 th Place	City Seattle	State & Zip Code/Country Washington, 98117/USA
Signature of Inventor 202:			Date:	
203	Full Name Of Inventor	Family Name Bajorath	First Given Name Jurgen	Second Given Name
	Residence & Citizenship	City Lynnwood	State or Foreign Country Washington	Country of Citizenship Germany
	Post Office Address	Post Office Address 17406 37 th Avenue W.	City Lynnwood	State & Zip Code/Country Washington, 98037/USA
Signature of Inventor 203:			Date: 01-03-05	

2	Full Name Of Inventor	Family Name Peach	First Given Name Robert	Second Given Name J.
0	Residence & Citizenship	City San Diego	State or Foreign Country California	Country of Citizenship New Zealand
4	Post Office Address	Post Office Address 12848 Via Caballo Rojo	City San Diego	State & Zip Code/Country California 92129/ USA
Signature of Inventor 204:			Date: 12/26/04	
2	Full Name Of Inventor	Family Name Brady	First Given Name William	Second Given Name
0	Residence & Citizenship	City Bothell	State or Foreign Country Washington	Country of Citizenship USA
5	Post Office Address	Post Office Address 618 219 th Place S.W.	City Bothell	State & Zip Code/Country Washington, 98021/USA
Signature of Inventor 205:			Date:	
2	Full Name Of Inventor	Family Name Damle	First Given Name Nitin	Second Given Name
0	Residence & Citizenship	City Upper Saddle River	State or Foreign Country New Jersey	Country of Citizenship USA
6	Post Office Address	Post Office Address 53 Stevenson Lane	City Upper Saddle River	State & Zip Code/Country New Jersey, 07458/USA
Signature of Inventor 206:			Date:	

§ 1.56 Duty to disclose information material to patentability.

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

- (1) prior art cited in search reports of a foreign patent office in a counterpart application, and
- (2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

- (1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim;
- or
- (2) It refutes, or is inconsistent with, a position the applicant takes in:
 - (i) Opposing an argument of unpatentability relied on by the Office, or
 - (ii) Asserting an argument of patentability.

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2	Full Name Of Inventor	Family Name Brady	First Given Name William	Second Given Name
0	Residence & Citizenship	City Bothell	State or Foreign Country Washington	Country of Citizenship USA
5	Post Office Address	Post Office Address 618 219 th Place S.W.	City Bothell	State & Zip Code/Country Washington, 98021/USA
Signature of Inventor 205:			Date: 2/22/05	
2	Full Name Of Inventor	Family Name Damle	First Given Name Mitin	Second Given Name
0	Residence & Citizenship	City Upper Saddle River	State or Foreign Country New Jersey	Country of Citizenship USA
6	Post Office Address	Post Office Address 53 Stevenson Lane	City Upper Saddle River	State & Zip Code/Country New Jersey, 07458/USA
Signature of Inventor 206:			Date:	

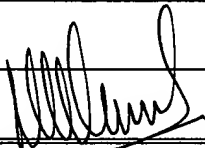
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6	Post Office Address	Post Office Address 53 Stevenson Lane	City Upper Saddle River	State & Zip Code/Country New Jersey, 07458/USA
Signature of Inventor 206: 				Date: 01-14-2005

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- (1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or
- (2) It refutes, or is inconsistent with, a position the applicant takes in:
 - (i) Opposing an argument of unpatentability relied on by the Office, or
 - (ii) Asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

(1) Each inventor named in the application:

(2) Each attorney or agent who prepares or prosecutes the application; and

(3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.

DKT. 30436.30US12/SBA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley, Jeffrey A. Ledbetter, Jorgen Bajorath, Robert J. Peach, William Brady, and Nitin Damle

Serial No. : 09/609,915 Examiner: Lorraine Spector, Ph.D.

Filed : July 3, 2000 Group Art Unit: 1647

Title : SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir/Madam:

WRITTEN CONSENT OF ASSIGNEE UNDER 37 C.F.R. 83.73(b)

Petitioner, the Bristol-Myers Squibb Company, a corporation organized and existing under the laws of the State of New Jersey and having its primary place of business at P.O. Box 4000, Lawrenceville-Princeton Road, Princeton, New Jersey 08453-4000, certifies that it is the assignee of the entire right, title and interest in the patent application identified above by virtue of assignments from the inventors for the subject application and related parent patent applications.

The subject application is: a continuation-in-part of U.S. Serial No. 08/228,208, filed April 15, 1994, now U.S. Patent No. 6,090,914, issued July 18, 2000, which was a continuation-in-part of U.S. Serial No. 08/008,898, filed January 22, 1993, now U.S. Patent No. 5,770,197, issued June 23, 1998, which was a continuation-in-part of U.S. Serial No. 07/723,617, filed June 27, 1991, now abandoned.

Applicants: Peter S. Linsley, et al.
U.S. Serial No: 09/609,915
Filed: July 3, 2000
Page: 2

The assignment for the parent patent applications in the chain of title for the subject application U.S. Serial No. 09/609,915 are recorded at the U.S. Patent and Trademark Office as follows:

- Reel 7169, Frame 0094, for U.S. Serial No. 08/228,208 (Exhibit 2A) except for the recorded assignments of Drs. Peach and Bajorath (attached herewith as Exhibit 2A-1 including the recordation form cover sheet)
- Reel 6567, Frame 0839, for U.S. Serial No. 08/008,898 (Exhibit 2B)
- Reel 5821, Frame 588, for U.S. Serial No. 07/723,617 (Exhibit 2C)

The assignments for the subject application have been executed by all the inventors except for Dr. Jeffrey Ledbetter (see attached as Exhibit 2D).

The undersigned has reviewed all the documents in the chain of title of the patent application identified above. Despite Dr. Ledbetter's refusal to execute an Assignment of Rights from himself to Bristol-Myers Squibb Company in the subject application and because Dr. Ledbetter's contributions to the claimed invention are disclosed in the parent applications to which the subject application claims priority and which parent applications Dr. Ledbetter has assigned to Bristol-Myers Squibb Company, to the best of undersigned's knowledge and belief, title is in the assignee identified above. However, in case the Patent Office disagrees, Applicants provide a Petition under 37 C.F.R. §1.183 requesting that the Patent Office suspend the rules regarding 37 C.F.R. §3.73(b) that ownership be established by documentary evidence of a chain of title from the original inventor (i.e. inventor Ledbetter) to assignee in the subject application and to accept the Written Consent of Assignee under 37 C.F.R. §3.73(b) (Exhibit 3).

The undersigned (whose title is provided below) is empowered to act on behalf of the assignee. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and

Applicants: Peter S. Linsley, et al.
U.S. Serial No: 09/609,915
Filed: July 3, 2000
Page: 3

further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 11/28/05

By:

Briana C. Bergen

Briana C. Bergen
Registration No. 39,123
Senior Counsel – Biotechnology Patents
Biotechnology Patents
Bristol-Myers Squibb Company
P.O. Box 4000
Princeton, New Jersey 08543-4000
Customer No. 23,914

MANDEL & ADRIANO

United States Patent Application

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

The specification of which

- a. ☐ is attached hereto
 b. ☒ was filed on July 3, 2000 as application serial no. 09/609,915 and was amended on April 15, 2002, May 8, 2002 (hand delivered May 9, 2002), September 9, 2002, November 4, 2002, June 2, 2004 and August 2, 2004 (resubmitted on August 23, 2004) (if applicable) (in the case of a PCT-filed application) described and claimed in international no. filed and as amended on , which I have reviewed and for which I solicit a United States patent.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

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- a. ☐ no such applications have been filed.
 b. ☐ such applications have been filed as follows:

FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC § 119			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)
ALL FOREIGN APPLICATION(S), IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S)			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)

I hereby claim the benefit under Title 35, United States Code, § 120/365 of any United States and PCT international application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. APPLICATION NUMBER	DATE OF FILING (day, month, year)	STATUS (patented, pending, abandoned)
07/723,617	27 June 1991	Abandoned
08/008,898	22 January 1993	Patented
08/228,208	15 April 1994	Patented

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

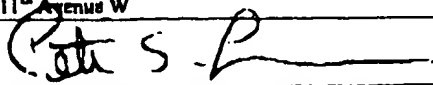
U.S. PROVISIONAL APPLICATION NUMBER	DATE OF FILING (Day, Month, Year)

I hereby appoint the following attorneys and agents associated with Customer No. 23914, respectively and individually, as my attorneys and agents, with full power of substitution, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Please direct all communication to the address associated with Customer No. 23914, which is currently:

Stephen B. Davis
Bristol-Myers Squibb Company
Patent Department
P.O. Box 4000
Princeton, New Jersey 08543-4000

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2	Full Name Of Inventor	Family Name Linsley	First Given Name Pam	Second Given Name S.
0	Residence & Citizenship	City Seattle	State or Foreign Country Washington	Country of Citizenship USA
1	Post Office Address	Post Office Address 2528 11 th Avenue W	City Seattle	State & Zip Code/Country Washington, 98119/USA
Signature of Inventor 201: 				Date: 01/03/04 ^{US} _{PS} 112805
2	Full Name Of Inventor	Family Name Ledbetter	First Given Name Jeffrey	Second Given Name A.
0	Residence & Citizenship	City Seattle	State or Foreign Country Washington	Country of Citizenship USA
1	Post Office Address	Post Office Address 306 N.W. 113 th Place	City Seattle	State & Zip Code/Country Washington, 98117/USA
Signature of Inventor 202:				Date:
2	Full Name Of Inventor	Family Name Bajorath	First Given Name Jurgen	Second Given Name
0	Residence & Citizenship	City Lynnwood	State or Foreign Country Washington	Country of Citizenship Germany
3	Post Office Address	Post Office Address 17406 37 th Avenue W.	City Lynnwood	State & Zip Code/Country Washington, 98037/USA
Signature of Inventor 203:				Date:

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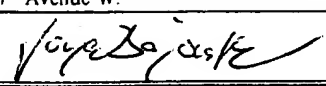
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Signature of Inventor 203: 			Date: 01-03-05	

2	Full Name Of Inventor	Family Name Peach	First Given Name Robert	Second Given Name J.
0	Residence & Citizenship	City San Diego	State or Foreign Country California	Country of Citizenship New Zealand
4	Post Office Address	Post Office Address 12848 Via Caballo Rojo	City San Diego	State & Zip Code/Country California 92129/ USA
Signature of Inventor 204:			Date: 12/26/04	
2	Full Name Of Inventor	Family Name Brady	First Given Name William	Second Given Name
0	Residence & Citizenship	City Bothell	State or Foreign Country Washington	Country of Citizenship USA
5	Post Office Address	Post Office Address 618 219 th Place S.W.	City Bothell	State & Zip Code/Country Washington, 98021/USA
Signature of Inventor 205:			Date:	
2	Full Name Of Inventor	Family Name Damle	First Given Name Nitin	Second Given Name
0	Residence & Citizenship	City Upper Saddle River	State or Foreign Country New Jersey	Country of Citizenship USA
6	Post Office Address	Post Office Address 53 Stevenson Lane	City Upper Saddle River	State & Zip Code/Country New Jersey, 07458/USA
Signature of Inventor 206:			Date:	

§ 1.56 Duty to disclose information material to patentability.

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

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- (2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

- (1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim;
- or
- (2) It refutes, or is inconsistent with, a position the applicant takes in:
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 - (ii) Asserting an argument of patentability.

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Signature of Inventor 205:			Date: 2/22/05	
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 - (i) Opposing an argument of unpatentability relied on by the Office, or
 - (ii) Asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

(1) Each inventor named in the application:

(2) Each attorney or agent who prepares or prosecutes the application; and

(3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.

MANDEL & ADRIANO

INTELLECTUAL PROPERTY ATTORNEYS

SARALYNN MANDEL
SARAH B. ADRIANO*
55 S. LAKE AVENUE, SUITE 710
PASADENA, CA 91101
PHONE 626/395-7801
FAX 626/395-0694
*Admitted in New York Only

December 23, 2004

By Federal Express
Dr. Jeffrey A. Ledbetter
306 N.W. 113th Place
Seattle, Washington, 98117

Re: Peter S. Linsley et al., entitled "SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF," U.S. Serial No. 09/609,915 filed July 3, 2000, which is a CIP of U.S. Serial No. 08/228,208 filed April 15, 1994, now U.S. Patent No. 6,090,914 issued on July 18, 2000, which was a CIP of U.S. Serial No. 08/008,898 filed January 22, 1993, now U.S. Patent No. 5,770,197 issued on June 23, 1998, which was a CIP of U.S. Serial No. 07/723,617 filed June 27, 1991, now abandoned; BMS Ref. ON0085N-US-CIP; M&A Ref. 30436.30USI2

Dear Dr. Ledbetter:

We enclose a Combined Declaration and Power of Attorney (Exhibit 1), in connection with the above-identified application. We also enclose copies of: the originally-filed patent application (Exhibit 2) and the Amendments filed in connection with the subject application on April 15, 2002 (Exhibit 2a), May 8, 2002 (hand delivered May 9, 2002) (Exhibit 2b), September 9, 2002 (Exhibit 2c), November 4, 2002 (Exhibit 2d), June 2, 2004 (Exhibit 2e) and August 2, 2004 (Exhibit 2f) (resubmitted on August 23, 2004 (Exhibit 2g)); and pending claims 1-30 (Exhibit 3).

The originally-filed claims were directed to **soluble CTLA4 mutant molecules**. As originally-filed, the named inventors were Peter Linsley, Jeffrey Ledbetter, Nitin Damle, William Brady, Jurgen Bajorath, Robert Peach, and Philip Wallace. Dr. Wallace was originally included in the inventorship list, based on his inventive contribution in one of the earlier patent applications, to which the subject application claims priority. However, upon further consideration, we have concluded that Dr. Philip Wallace is not an inventor of any of the originally-filed claims directed to soluble CTLA4 mutant molecule of this case. Accordingly, the inventorship of the subject application must be changed to exclude

Dr. Jeffrey A. Ledbetter
December 23, 2004
Page 2

Dr. Wallace as an inventor. If you disagree with our assessment of inventorship, please contact us immediately.

In order to effect a change of inventorship, please review again the enclosed application (Exhibit 2) and amendments (Exhibits 2a-2g) for accuracy, and if accurate then review and sign and date the Combined Declaration and Power of Attorney (Exhibit 1) where indicated, in black ink.

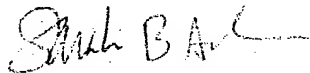
Please return the signed and dated Combined Declaration and Power of Attorney (Exhibit 1) to us by facsimile, and the originals by mail to the above address before:

JANUARY 23, 2005

Failure to return the executed documents by the **JANUARY 23, 2005** date will be viewed as a refusal to sign.

Please contact our office if you have any questions or comments. Thank you for your cooperation.

Sincerely,



Sarah B. Adriano

SBA/RDG/HVR/rapd
Enclosures

cc: Audrey F. Sher, Esq. (w/o encl.)
Roberta D. German, Ph.D. (w/o encl.)
Hema Vakharia-Rao, Ph.D. (w/o encl.)

m:\bristol\30usi2\corres clients\petition letters\04 dec 22 inventor's dec-poa ledbetter.doc

MANDEL & ADRIANO

United States Patent Application

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

The specification of which

- a. ☐ is attached hereto
 b. ☒ was filed on July 3, 2000 as application serial no. 09/609,915 and was amended on April 15, 2002, May 8, 2002 (hand delivered May 9, 2002), September 9, 2002, November 4, 2002, June 2, 2004 and August 2, 2004 (resubmitted on August 23, 2004) (if applicable) (in the case of a PCT-filed application) described and claimed in international no. filed and as amended on , which I have reviewed and for which I solicit a United States patent.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56 (attached hereto).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on the basis of which priority is claimed:

- a. ☐ no such applications have been filed.
 b. ☐ such applications have been filed as follows:

FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC § 119			
COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)
ALL FOREIGN APPLICATION(S), IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S)			
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U.S. APPLICATION NUMBER	DATE OF FILING (day, month, year)	STATUS (patented, pending, abandoned)
07/723,617	27 June 1991	Abandoned
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2 0 1	Full Name Of Inventor	Family Name Linsley	First Given Name Peter	Second Given Name S.
	Residence & Citizenship	City Seattle	State or Foreign Country Washington	Country of Citizenship USA
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Signature of Inventor 201:				Date:
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(3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

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SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

5 This application is a continuation-in-part of U.S. Serial No. 08/228,208, filed April 15, 1994,
U.S. Serial No. 08/539,436, filed October 19, 1995, and U.S. Serial No. not yet known, filed
June 26, 2000, which is a continuation in part of U.S. Serial No. 09/014,761, filed January 28,
1998, which claims priority of U.S. Serial No. 60/036,549, filed January 28, 1997, now
abandoned, the contents of all of which are incorporated by reference into the present
10 application.

Throughout this application various publications are referenced. The disclosures of these
publications in their entireties are hereby incorporated by reference into this application in order
to more fully describe the state of the art to which this invention pertains.

15

FIELD OF THE INVENTION

The present invention relates to the field of soluble CTLA4 molecules which are mutated from
its wildtype counterpart and binds CD80 and/or CD86.

20

BACKGROUND OF THE INVENTION

Antigen-nonspecific intercellular interactions between T-lymphocytes and antigen-presenting
cells (APCs) generate T cell stimulatory signals that generate T cell responses to antigen
25 (Jenkins and Johnson 1993 *Curr. Opin. Immunol.* 5:361-367). Stimulatory signals determine
the magnitude of a T cell response to antigen, and whether this response activates or
inactivates subsequent responses to antigen (Mueller et al. 1989 *Annu. Rev. Immunol.* 7: 445-
480).

30 T cell activation in the absence of costimulation results in an aborted or anergic T cell
response (Schwartz, R.H. 1992 *Cell* 71:1065-1068). One key stimulatory signal is provided

by interaction of T cell surface receptors CD28 and CTLA4 with B7 related molecules on APC (e.g., also known as B7-1 and B7-2, or CD80 and CD86, respectively) (P. Linsley and J. Ledbetter 1993 *Annu. Rev. Immunol.* 11:191-212).

- 5 The molecule now known as CD80 (B7-1) was originally described as a human B cell-associated activation antigen (Yokochi, T. et al. 1981 *J. Immunol.* 128:823-827; Freeman, G.J. et al. 1989 *J. Immunol.* 143:2714-2722), and subsequently identified as a counterreceptor for the related T cell molecules CD28 and CTLA4 (Linsley, P., et al. 1990 *Proc. Natl. Acad. Sci. USA* 87:5031-5035; Linsley, P.S. et al. 1991(a) *J. Exp. Med.* 173:721-730; Linsley, P.S. et al. 1991(b) *J. Exp. Med.* 174:561-570).

- More recently, another counterreceptor for CTLA4 was identified on antigen presenting cells (APC) (Azuma, N. et al. 1993 *Nature* 366:76-79; Freeman 1993(a) *Science* 262:909-911; Freeman, G.J. et al. 1993(b) *J. Exp. Med.* 178:2185-2192; Hathcock, K.L.S., et al. 1994 *J. Exp. Med.* 180:631-640; Lenschow, D.J. et al., 1993 *Proc. Natl. Acad. Sci. USA* 90:11054-11058; Ravi-Wolf, Z., et al. 1993 *Proc. Natl. Acad. Sci. USA* 90:11182-11186; Wu, Y. et al. 1993 *J. Exp. Med.* 178:1789-1793).

- This molecule, now known as CD86 (Caux, C., et al. 1994 *J. Exp. Med.* 180:1841-1848), but also called B7-0 (Azuma et al., 1993, *supra*) or B7-2 (Freeman et al., 1993a, *supra*), shares about 25% sequence identity with CD80 in its extracellular region (Azuma et al., 1993, *supra*; Freeman et al., 1993a, *supra*, 1993b, *supra*). CD86-transfected cells trigger CD28-mediated T cell responses (Azuma et al., 1993, *supra*; Freeman et al., 1993a, 1993b, *supra*).

- 25 Comparisons of expression of CD80 and CD86 have been the subject of several studies (Azuma et al. 1993, *supra*; Hathcock et al., 1994 *supra*; Larsen, C.P., et al. 1994 *J. Immunol.* 152:5208-5219; Stack, R.M., et al., 1994 *J. Immunol.* 152:5723-5733). Current data indicate that expression of CD80 and CD86 are regulated differently, and suggest that CD86 expression tends to precede CD80 expression during an immune response.

Soluble forms of CD28 and CTLA4 have been constructed by fusing variable (v)-like extracellular domains of CD28 and CTLA4 to immunoglobulin (Ig) constant domains resulting in CD28Ig and CTLA4Ig. CTLA4Ig binds both CD80 positive and CD86 positive cells more strongly than CD28Ig (Linsley, P. et al. 1994 *Immunity* 1:793-80). Many T cell-dependent immune responses are blocked by CTLA4Ig both *in vitro* and *in vivo*. (Linsley, et al., (1991b), *supra*; Linsley, P.S. et al., 1992(a) *Science* 257:792-795; Linsley, P. S. et al., 1992(b) *J. Exp. Med.* 176:1595-1604; Lenschow, D.J. et al. 1992, *Science* 257:789-792; Tan, P. et al., 1992 *J. Exp. Med.* 177:165-173; Turka, L.A., 1992 *Proc. Natl. Acad. Sci. USA* 89:11102-11105).

Soluble CTLA4 molecules are effective immunosuppressive agents. There is a need to discover additional soluble CTLA4 molecules for treatments requiring donor-specific and antigen-specific tolerance.

SUMMARY OF THE INVENTION

The invention provides soluble CTLA4 mutant molecules that bind CD80 and/or CD86. In accordance with the practice of this invention, soluble CTLA4 molecules of the invention have amino acid changes in the extracellular domain of *CTLA4* so as to produce molecules which would retain the functional property of CTLA4, namely, the mutant molecule will still bind either CD80, CD86, or both. In some embodiments, certain mutant molecules bind CD80 and/or CD86 with greater or similar avidity, compared to CTLA4.

CTLA4 mutant molecules comprise the extracellular domain of CTLA4 having an amino acid residue(s) replaced with another amino acid(s). The replacement amino acid residue can be any of the 20 natural amino acids or a non-naturally occurring amino acid. Embodiments of the mutant molecule include molecules having a single amino acid substitution at position S25, P26, G27, K28, A29, T30, E31, or R33. Other embodiments include mutant molecules having a single amino acid substitution at position K93, L96, M97, Y98, P99, P100, P101, Y102, or Y103. Additional embodiments includes mutant molecules having a single amino acid substitution at position L104, G105, I106, G107, Q111, Y113, or I115.

Examples of CTLA4 mutant molecules which bind CD86 more avidly than wildtype, e.g., CTLA4Ig, include certain mutants having amino acid substitutions at position S25, A29, T30, K93, L96, Y103, L104, or G105.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Shows a schematic representation of a CTLA4Ig fusion construct as described in Example 1, infra.

Figure 2: Shows a photograph of a gel obtained from SDS-PAGE chromatographic purification of CTLA4Ig as described in Example 1, infra.

Figure 3: Shows the complete amino acid sequence encoding human CTLA4 receptor fused to the oncostatin M signal peptide, and including the newly identified N-linked glycosylation site, as described in Example 1, infra.

Figure 4: Depicts the results of FACS^R analysis of binding of the B7Ig fusion protein to CD28- and CTLA4-transfected COS cells as described in Example 4, infra.

Figure 5: Depicts the results of FACS^R analysis of binding of purified CTLA4Ig on B7 antigen-positive (B7⁺) CHO cells and on a lymphoblastoid cell line (PM LCL) as described in Example 4, infra.

Figure 6: Shows a graph illustrating competition binding analysis of ¹²⁵I labeled B7Ig to immobilized CTLA4Ig as described in Example 4, infra.

Figure 7: Depicts a graph showing the results of Scatchard analysis of ¹²⁵I-labeled B7Ig binding to immobilized CTLA4Ig as described in Example 4, infra.

Figure 8: Shows a photograph of a gel from SDS-PAGE chromatography of immunoprecipitation analysis of B7 positive CHO cells and PM LCL cells surface-labeled with ^{125}I as described in Example 4, infra.

5 Figure 9: Depicts a graph showing the effects on proliferation of T cells of CTLA4Ig as measured by [^3H]-thymidine incorporation as described in Example 4, infra.

Figure 10: Shows a bar graph illustrating the effects of CTLA4Ig on helper T cell (T_h)-induced immunoglobulin secretion by human B cells as determined by enzyme immunoassay (ELISA) as
10 described in Example 4, infra.

Figure 11a-c: Depicts graphs showing the survival of human pancreatic islet xenografts in mice, as described in Example 5, infra.

- A) Control animals treated with PBS (solid lines) or L6 (dotted lines);
15 B) Animals treated with CTLA4Ig for 14 consecutive days immediately after human islet transplantation;
C) Animals treated with CTLA4Ig every other day for 14 days immediately after human islet transplantation.

20 Figures 12: Shows photographs of histopathology slides of human islets transplanted under the kidney capsule of B10 mice. A) Hematoxylin and eosin staining of human islet grafted in a control B10 mouse; B) The tissue shown in Figure A, stained for insulin; C) Hematoxylin and eosin staining of a human islet in a B10 grafted mouse treated with CTLA4; D) The tissue shown in Figure C, stained for insulin.

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Figure 13: Depicts a line graph showing the prolongation of islet graft survival with MAb to human B7 in streptozotocin-treated animals.

Figure 14: Depicts a line graph showing induction of donor-specific unresponsiveness to islet
30 graft antigens by CTLA4Ig.

Figure 15: Depicts a graph showing antibody serum titer levels of mice injected with sheep red blood cells (SRBC), mAb L6 and rat Ig, mAb L6 and anti-IL4, CTLA4Ig and rat Ig, CTLA4Ig and anti-IL4. The X axis measures the antibody-serum titer. The Y axis measures time in days. The closed box represents mice injected with SRBC at day 0 and day 46. The open box represents mice injected with SRBC at day 46. The closed circle represents mice injected with mAb L6 and rat immunoglobulin. The open circle represents mice injected with mAb L6 and anti-IL4 antibody. The closed triangle represents mice injected with CTLA4Ig and rat immunoglobulin. The open triangle represents mice injected with CTLA4Ig and anti-IL4 antibody.

Figure 16: Depicts a graph showing antibody serum titer levels of mice injected with KLH, mAb L6 and rat Ig, mAb L6 and anti-IL4, CTLA4Ig and rat Ig, CTLA4Ig and anti-IL4. The X axis measures the antibody-serum titer. The Y axis measures time in days. The closed box represents mice injected with keyhole limpet hemocyanin (KLH) at day 46. The closed circle represents mice injected with mAb L6 and rat immunoglobulin. The open circle represents mice injected with mAb L6 and anti-IL4 antibody. The closed triangle represents mice injected with CTLA4Ig and rat immunoglobulin. The open triangle represents mice injected with CTLA4Ig and anti-IL4 antibody.

Figure 17: Depicts sequencing alignment of CD28 and CTLA4 family members. Sequences of human (H), mouse (M), rat (R), and chicken (Ch) CD28 are aligned with human and mouse CTLA4. The signal peptides are underlined with a dashed line. The transmembrane domains are underlined with a solid line. The CDR-analogous regions are noted. The dark shaded areas highlight complete conservation of residues while the light shaded areas highlight conservative amino acid substitutions in all family members.

Figure 18: Depicts a graph showing the results of a binding assay of CTLA4Ig mutants with B7-1. The various CTLA4Ig mutants each have a residue within the MYPPPY motif replaced with an alanine, as described in Example 7, infra.

Figure 19: Shows a schematic map of CTLA4/CD28Ig hybrid fusion proteins. Open areas represent CD28 sequence; solid areas represent CTLA4 sequence; cross-hatched areas represent beginning of IgG Fc (also refer to Example 7, infra).

5 Figures 20a-b: Depicts graphs showing the results of a binding assay of CTLA4/CD28 hybrid fusion molecules and B7-1.

A) A comparison of the binding activity of CTLA4Ig, CD28Ig, HS2, HS4, and HS6;

B) A comparison of the binding activity of CTLA4Ig, CD28Ig, HS5, HS4-43, and HS8.

10 Figure 21: A molecular model of monomeric CTLA4Ig v-like extracellular domain.

Figure 22: Depicts the amino acid sequence of a CTLA4Ig having wildtype extracellular domain of CTLA4.

15 Figure 23: Depicts the nucleotide and amino acid sequences of L104EIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.

Figure 24: Depicts the nucleotide and amino acid sequence of L104EA29YIg starting at
20 methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.

Figure 25: Depicts the nucleotide and amino acid sequences of L104EA29LIg starting at
25 methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.

Figure 26: Depicts the nucleotide and amino acid sequences of L104EA29TIg starting at
methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to
aspartic acid at position +124.

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Figure 27: Depicts the nucleotide and amino acid sequences of L104EA29YIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.

- 5 Figure 28: Depicts the results of an equilibrium binding analysis of L104EA29YIg, L104EIg, and wild-type CTLA4Ig to CD86Ig. L104EA29YIg binds more strongly to CD86Ig than does L104EIg or CTLA4Ig. Equilibrium binding constants (K_d) were determined and shown Example 9, *infra*. The lower K_d of L104EA29YIg (3.21) than L104EIg (6.06) or CTLA4Ig (13.9) indicates higher binding avidity to CD86Ig. The lower K_d of L104EA29YIg (3.66) than L104EIg (4.47) or CTLA4Ig (6.51) indicates higher binding avidity to CD80Ig.
- 10

- Figure 29: Depicts the results of a FACS assay, showing L104EA29YIg and L104EIg bind more strongly to CHO cells stably transfected with human CD86 than does CTLA4Ig. A) L104EA29YIg and L104EIg binding to human CD80 CHO-transfected cells; B) L104EA29YIg and L104EIg binding to human CD86 CHO-transfected cells.
- 15

- Figure 30: Depicts the results of *in vitro* functional assays showing that L104EA29YIg is ~10-fold more effective than CTLA4Ig at inhibiting proliferation of CD86 + PMA treated human T cells. Inhibition of CD80 + PMA stimulated proliferation by CTLA4Ig and L104EA29YIg is more equivalent. A) L104EA29YIg inhibits proliferation of CD80 + PMA treated human T cells; B) L104EA29YIg inhibits proliferation of CD86 + PMA treated human T cells.
- 20

- Figure 31: Depicts the results of *in vitro* functional assays, showing L104EA29YIg is approximately 10-fold more effective than CTLA4Ig at inhibiting proliferation of primary and secondary allostimulated T cells. A) The effect of L103EA29YIg on primary allostimulated T cells; B) The effect of L103EA29YIg on secondary allostimulated T cells.
- 25

- Figure 32: Depicts the results of *in vitro* functional assays, showing L104EA29YIg is 5-7-fold more effective than CTLA4Ig at inhibiting IL-2, IL-4, and γ -interferon cytokine production of allostimulated human T cells. A) The effect of L104EA29YIg on IL-2
- 30

production; B) The effect of L104EA29YIg on IL-4 production; C) The effect of L104EA29YIg on gamma-IFN production.

5 Figure 33: Depicts the results of *in vitro* functional assays, showing L104EA29YIg is ~10-fold more effective than CTLA4Ig at inhibiting proliferation of PHA-stimulated monkey PBMC's.

10 Figure 34: Depicts the results of *in vitro* functional assays, showing inhibition of proliferation of T cells stimulated with PMA and CD80-CHO or CD86-CHO cells. A) The inhibitory effect of L104EIg and L104SIg on T cells stimulated with PMA blasts and CD80-CHO cells; B) the inhibitory effect of L104DIg and L104SIg on T cells stimulated with PMA blasts and CD80-CHO cells.

15 Figure 35: Depicts the results of a FACS assay, showing L104EIg and L104EG105FIg bind CHO cells stably transfected with human CD80 or CD86. A) L104EIg and L104EG105FIg bind to human CD80 CHO-transfected cells; B) L104EIg and L104E105FIg bind to human CD86 CHO-transfected cells.

20 Figure 36: Depicts the results of *in vitro* functional assays, showing L104EIg and L104EG105FIg inhibit proliferation of primary allostimulated T cells.

25 Figure 37: Figure 35: Depicts the results of a FACS assay, showing L104EIg and L104ES25RIg bind CHO cells stably transfected with human CD80 or CD86. A) L104EIg and L104ES25RIg bind to human CD80 CHO-transfected cells; B) L104EIg and L104ES25RIg bind to human CD86 CHO-transfected cells.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

30

As used in this application, the following words or phrases have the meanings specified.

As used herein a "CTLA4 mutant molecule" is a molecule including full length CTLA4 or portions thereof (e.g., fragments) having the activity of binding CD80 and/or CD86 and that has a mutation or multiple mutations in the extracellular domain of CTLA4, so that the sequence of the mutant molecule is not identical to the wildtype CTLA4 molecule. The CTLA4 mutant molecules may be fusion molecules comprising a non-CTLA4 molecule attached thereto. The mutant molecules may be soluble (i.e., circulating) or bound to a surface. CTLA4 mutant molecules can be made synthetically or recombinantly.

As used herein, the term "mutation" means a change in the amino acid sequence of the wild-type CTLA4 extracellular domain. The amino acid changes include substitutions, deletions, insertions, additions, or truncations. The mutant molecule can have one or more mutations.

As used herein "the extracellular domain of CTLA4" is the portion of the CTLA4 receptor that extends outside the cell membrane or any portion thereof which recognizes and binds CD80 and/or CD86.

As used herein "CTLA4" has the sequence of wildtype, full length CTLA4 as shown in Figure 3 of U.S. Patent Nos. 5,434,131, 5,844,095, 5,851,795, or any portion thereof which binds CD80 or CD86 or interferes with CD80 or CD86 so that it blocks binding to CD28 or CTLA4 (e.g., endogenous CD28 or CTLA4). CTLA4 is a cell surface protein, having an N-terminal extracellular domain, a transmembrane domain, and a C-terminal cytoplasmic domain. The extracellular domain binds to target antigens, such as CD80 or CD86. In a cell, the naturally occurring, wild type CTLA4 protein is translated as an immature polypeptide, which includes a signal peptide at the N-terminal end. The immature polypeptide undergoes post-translational processing, which includes cleavage and removal of the signal peptide to generate a CTLA4 cleavage product having a newly generated N-terminal end that differs from the N-terminal end in the immature form. One skilled in the art will appreciate that additional post-translational processing may occur, which removes one or more of the amino acids from the newly generated N-terminal end of the CTLA4 cleavage product. The mature form of the CTLA4 molecule includes the extracellular domain or any portion thereof which binds to CD80 and/or CD86.

One embodiment of a soluble CTLA4 has been deposited with the American Type Culture Collection (ATCC) in Manassas, Maryland, under the provisions of the Budapest Treaty on May 31, 1991 and has been accorded ATCC accession number: 68629. ATCC 68629 is DNA encoding *CTLA4* Ig. Additionally, the CTLA4Ig-24 CHO cell line has been deposited with the ATCC under the Budapest Treaty on May 31, 1991 and has been accorded accession number ATCC 10762. DNA encoding L104EA29YIg was submitted for deposit on June 19, 2000 with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209. The ATCC accession number has not yet been assigned.

10 As used herein a "non-CTLA4 protein sequence" or "non-CTLA4 molecule" means any protein molecule which does not bind CD80 and/or CD86 and does not interfere with the binding of CTLA4 to its target. An example includes, but is not limited to, an immunoglobulin (Ig) constant region or portion thereof. Preferably, the Ig constant region is a human or monkey Ig constant region, e.g., human C(gamma)1, including the hinge, CH2 and CH3 regions. The Ig
15 constant region can be mutated to reduce its effector functions (U.S. Patent Nos: 5,844,095; 5,851,795; and 5,885,796).

As used herein a "fragment" is any portion of CTLA4 mutant molecule, preferably the extracellular domain of CTLA4 or a portion thereof that recognizes and binds its target, e.g.,
20 CD80 and/or CD86.

As used herein "blocks T cell proliferation" means to bind CD80 or CD86, e.g., on APCs, so that T cell proliferation is detectably inhibited by an art recognized test such as by nucleotide
25 incorporation into DNA or clonogenic assay.

As used herein "blocking B7 interaction" means to interfere with the binding of B7 to its ligands such as CD28 and/or CTLA4 thereby obstructing T cell and B cell interaction.

30 As used herein "regulating functional *CTLA4* positive T cell interaction" means to suppress an immune response directly or indirectly.

As used herein "at least a portion" means any part of the molecule which recognizes and binds its target, e.g., CD80 or CD86.

5 As used herein "immunoproliferative disease" means any disease mediated by T cell interactions with CD80 or CD86 positive cells including but not limited to graft versus host disease (GVHD); psoriasis; immune disorders associated with graft transplantation rejection; T cell lymphoma; T cell acute lymphoblastic leukemia; testicular angiocentric T cell lymphoma; benign lymphocytic angiitis; and autoimmune diseases such as lupus erythematosus, Hashimoto's thyroiditis, primary
10 myxedema, Graves' disease, pernicious anemia, autoimmune atrophic gastritis, Addison's disease, insulin dependent diabetes mellitus, good pasture's syndrome, myasthenia gravis, pemphigus, Crohn's disease, sympathetic ophthalmia, autoimmune uveitis, multiple sclerosis, autoimmune hemolytic anemia, idiopathic thrombocytopenia, primary biliary cirrhosis, chronic action hepatitis, ulceratis colitis, Sjogren's syndrome, rheumatoid arthritis, polymyositis,
15 scleroderma, and mixed connective tissue disease.

In order that the invention herein described may be more fully understood, the following description is set forth.

20 COMPOSITIONS OF THE INVENTION

The present invention provides soluble CTLA4 mutant molecules which recognize and bind CD80 and/or CD86. In some embodiments, the CTLA4 mutant molecules have similar or lower
25 avidity to CD80 and/or CD86 than CTLA4Ig. The preferred embodiment includes soluble CTLA4 mutants having a higher avidity to CD80 and/or CD86 than CTLA4Ig. The preferred mutant molecules should be better able to interfere or disrupt the priming of antigen specific activated cells than CTLA4Ig

30 The present invention provides CTLA4 mutant molecules comprising at least the extracellular domain of CTLA4 or portions thereof that bind CD80 and/or CD86. The extracellular portion of CTLA4 comprises methionine at position +1 through aspartic acid at position +124 (e.g., Figure

3 or 22). The extracellular portion of the CTLA4 can comprise alanine at position -1 through aspartic acid at position +124 (e.g., Figure 3 or 22). The extracellular portion of the CTLA4 can comprise glutamic acid at position +95 through cysteine at position +120. The extracellular portion of the CTLA4 can comprise methionine at position +1 through cysteine at position +21 and glutamic acid at position +95 through aspartic acid at position +122. The extracellular portion of the CTLA4 can comprise methionine at position +1 through tyrosine at position +23 and valine at position +32 through aspartic acid at position +122. The extracellular portion of the CTLA4 can comprise alanine at position +24 through glutamic acid at position +31 and glutamic acid at position +95 through aspartic acid at position +122. The extracellular portion of the CTLA4 can comprise alanine at position +24 through glutamic acid at position +31 and glutamic acid at position +95 through isoleucine at position +112. The extracellular portion of the CTLA4 can comprise alanine at position +24 through glutamic acid at position +31 and tyrosine at position +113 through aspartic acid at position +122. The extracellular portion of the CTLA4 can comprise alanine at position +50 through glutamic acid at position +57 and glutamic acid at position +95 through aspartic acid at position +122. The extracellular portion of the CTLA4 can comprise alanine at position +24 through glutamic acid at position +31; alanine at position +50 through glutamic acid at position +57; and glutamic acid at position +95 through aspartic acid at position +122. The extracellular portion of the CTLA4 can comprise alanine at position +50 through glutamic acid at position +57 and glutamic acid at position +95 through isoleucine at position +112. The extracellular portion of the CTLA4 can comprise alanine at position +24 through glutamic acid at position +31; alanine at position +50 through glutamic acid at position +57; and glutamic acid at position +95 through aspartic acid at position +122. The extracellular portion of CTLA4 can comprise alanine at position +24 through valine at position +94. The extracellular portion of CTLA4 can comprise alanine at position -1 through cysteine at position +21. The extracellular portion of CTLA4 can comprise methionine at position +1 through cysteine at position +21. The extracellular portion of CTLA4 can comprise glutamic acid at position +95 through aspartic acid at position +122. The extracellular portion of CTLA4 can comprise alanine at position -1 through valine at position +94. The extracellular portion of CTLA4 can comprise methionine at position +1 through valine at position +94. The extracellular portion of CTLA4 can comprise alanine at position +24 through glutamic acid at position +31. The extracellular portion of CTLA4 can comprise alanine at position -1 through

tyrosine at position +23. The extracellular portion of CTLA4 can comprise methionine at position +1 through tyrosine at position +23. The extracellular portion of CTLA4 can comprise valine at position +32 through aspartic acid at position +122. The extracellular portion of CTLA4 can comprise tyrosine at position +113 through aspartic acid at position +122. The extracellular portion of CTLA4 can comprise glutamic acid at position +95 through isoleucine at position +112. The extracellular portion of CTLA4 can comprise alanine at position +50 through glutamic acid at position +57.

In one embodiment, the soluble CTLA4 mutant molecules comprise one or more mutations (e.g., amino acid substitutions, deletions, or insertions) in the extracellular domain of CTLA4.

For example, the soluble CTLA4 mutant molecules can include a mutation or mutations within or in close proximity to the region encompassed by serine at position +25 through arginine at position +33 (e.g., S25-R33). The mutant CTLA4 molecules can include an amino acid substitution at any one or more of the following positions: S25, P26, G27, K28, A29, T30, E31, or R33.

In another embodiment, the soluble CTLA4 mutant molecules can include a mutation or mutations within or in close proximity to the region encompassed by glutamic acid at position +95 to glycine at position +107 (e.g., E95-G107). The mutant CTLA4 molecules can include an amino acid substitution at any one or more of the following positions: K93, L96, M97, Y98, P99, P100, P101, Y102, Y103, L104, G105, I106, and G107.

Additionally, the invention provides soluble CTLA4 mutant molecules having a mutation or mutations within or in close proximity to the region encompassed by asparagine +108 to isoleucine at position +115 (e.g., N108-I115). The mutant CTLA4 molecule can include an amino acid substitution at any one or more of the following positions: L104, G105, I106, G107, Q111, Y113, or I115. Examples of CTLA4 molecules having mutations in the CTLA4 extracellular domain are included in Tables 2, 3, and 4.

Additionally, the invention provides mutant molecules having one mutation in the extracellular domain of CTLA4. Examples include the following:

Single-site mutant:	Codon change:
L104EIg	Glutamic acid GAG
L104SIg	Serine AGT
L104TIg	Threonine ACG
L104AIg	Alanine GCG
L104Wlg	Tryptophan TGG
L104QIg	Glutamine CAG
L104Klg	Lysine AAG
L104RIg	Arginine CGG
L104GIg	Glycine GGG

5

Further, the invention provides mutant molecules having two mutations in the extracellular domain of CTLA4. Examples include the following:

Double-site mutants:	Codon change:
L104EG105FIg	Phenylalanine TTC
L104EG105Wlg	Tryptophan TGG
L104EG105LIg	Leucine CTT
L104ES25RIg	Arginine CGG
L104ET32GIg	Glycine GGG
L104ET32NIg	Asparagine AAT
L104EA29YIg	Tyrosine TAT
L104EA29LIg	Leucine TTG
L104EA29Tig	Threonine ACT
L104EA29Wlg	Tryptophan TGG

Further still, the invention provides mutant molecules having three mutations in the extracellular domain of CTLA4. Examples include the following:

Triple-site Mutants:	Codon changes:
L104EA29YS25KIg	Lysine AAA
L104EA29YS25KIg	Lysine AAG
L104EA29YS25NIg	Asparagine AAC
L104EA29YS25RIg	Arginine CGG

5

The invention additionally provides soluble CTLA4 mutant molecules comprising an extracellular domain of mutant CTLA4 and a moiety that alters the solubility, affinity and/or valency of the CTLA4 mutant molecule for binding CD80 and/or CD86.

10

In accordance with a practice of the invention, the moiety can be an immunoglobulin constant region or portion thereof. For in vivo use, it is preferred that the immunoglobulin constant region does not elicit a detrimental immune response in the subject. For example, in clinical protocols, it may be preferred that mutant molecules include human or monkey immunoglobulin constant regions. One example of a suitable immunoglobulin region is human C(gamma)1, comprising the hinge, CH2, and CH3 regions. Other isotypes are possible. Further, other immunoglobulin constant regions are possible (preferably other weakly or non-immunogenic immunoglobulin constant regions). The Ig can have one or more mutations therein, e.g., in the CH2 domain, to reduce effector functions such as CDC or ADCC.

15

20

In one embodiment, the soluble CTLA4 mutant molecule comprises the extracellular domain of CTLA4 joined to an immunoglobulin (Ig) portion, wherein the Ig has one or more mutations therein. The Ig portion can include the hinge, CH2, and CH3 regions which can mediate effector function of the soluble CTLA4 mutant molecule, such as binding to Fc receptors, mediating complement-dependent cytotoxicity (e.g., CDC), or mediate antibody-dependent cell-mediated cytotoxicity (e.g., ADCC). The mutation in the immunoglobulin

25

can modulate the binding capability of the immunoglobulin portion to its ligand, such as increase or decrease the binding capability of the immunoglobulin portion to the Fc receptors.

In a preferred embodiment, the soluble CTLA4 mutant molecule includes the immunoglobulin portion (e.g., hinge, CH2 and CH3 domains), where any or all of the cysteine residues, within the hinge domain are substituted with serine, for example, the cysteines at positions +130, +136, or +139 (Figure 1 or 22). The mutant molecule may also include the proline at position +148 substituted with a serine, as shown in Figure 22.

In another preferred embodiment, the soluble CTLA4 mutant molecule includes the immunoglobulin portion (e.g., hinge, CH2 and CH3 domains), having the leucine at position +144 substituted with phenylalanine, the leucine at position +145 substituted with glutamic acid, or glycine at position +147 substituted with alanine.

Other moieties include polypeptide tags. Examples of suitable tags include but are not limited to p97 molecule, env gp120 molecule, E7 molecule, and ova molecule (Dash, B., et al. 1994 *J. Gen. Virol.* 75:1389-97; Ikeda, T., et al. 1994 *Gene* 138:193-6; Falk, K., et al. 1993 *Cell. Immunol.* 150:447-52; Fujisaka, K. et al. 1994 *Virology* 204:789-93). Other molecules are possible (Gerard, C. et al. 1994 *Neuroscience* 62:721; Byrn, R. et al. 1989 63:4370; Smith, D. et al., 1987 *Science* 238:1704; Lasky, L., 1996 *Science* 233:209).

Soluble CTLA4 mutant molecules can have a junction amino acid residue which is located between the CTLA4 portion and the immunoglobulin portion of the molecule. The junction amino acid can be any amino acid, including glutamine. The junction amino acid can be introduced by molecular or chemical synthesis methods known in the art.

The present invention provides CTLA4 mutant molecules including a signal peptide sequence linked to the N-terminal end of the extracellular domain of the CTLA4 portion of the mutant molecule. The signal peptide can be any sequence that will permit secretion of the mutant molecule, including the signal peptide from oncostatin M (Malik, et al., 1989 *Molec. Cell. Biol.*

9: 2847-2853), or CD5 (Jones, N. H. et al., 1986 *Nature* 323:346-349), or the signal peptide from any extracellular protein.

5 The invention provides L104EIg (Figure 23) which is a mutant molecule including a CTLA4 portion encompassing methionine at position +1 through aspartic acid at position +124, a junction amino acid residue glutamine at position +125, and an immunoglobulin portion encompassing glutamic acid at position +126 through lysine at position +357. The portion having the extracellular domain of CTLA4 is mutated so that leucine at position +104 is substituted with glutamic acid. The hinge portion of the mutant molecule is mutated so that the
10 cysteines at positions +130, +136, and +139 are substituted with serine, and the proline at position +148 is substituted with serine. Alternatively, L104EIg can have a CTLA4 portion encompassing alanine at position -1 through aspartic acid at position +124.

15 The invention provides L104SIg which is a mutant molecule including a CTLA4 portion encompassing methionine at position +1 through aspartic acid at position +124, a junction amino acid residue glutamine at position +125, and an immunoglobulin portion encompassing glutamic acid at position +126 through lysine at position +357. The portion having the extracellular domain of CTLA4 is mutated so that leucine at position +104 is substituted with serine. The hinge portion of the mutant molecule is mutated so that the cysteines at positions +130, +136,
20 and +139 are substituted with serine, and the proline at position +148 is substituted with serine. Alternatively, L104SIg can have a CTLA4 portion encompassing alanine at position -1 through aspartic acid at position +124.

25 The invention provides L104EA29YIg (Figure 24) which is a mutant molecule including a CTLA4 portion encompassing methionine at position +1 through aspartic acid at position +124, a junction amino acid residue glutamine at position +125, and the immunoglobulin portion encompassing glutamic acid at position +126 through lysine at position +357. The portion having the extracellular domain of CTLA4 is mutated so that alanine at position +29 is substituted with tyrosine and leucine at position +104 is substituted with glutamic acid. The
30 immunoglobulin portion of the mutant molecule can be mutated, so that the cysteines at positions +130, +136, and +139 are substituted with serine, and the proline at position +148 is substituted

with serine. Alternatively, L104EA29YIg can have a CTLA4 portion encompassing alanine at position -1 through aspartic acid at position +124.

5 The invention provides L104EA29LIg (Figure 25) which is a mutant molecule including a CTLA4 portion encompassing methionine at position +1 through aspartic acid at position +124, a junction amino acid residue glutamine at position +125, and the immunoglobulin portion encompassing glutamic acid at position +126 through lysine at position +357. The portion having the extracellular domain of CTLA4 is mutated so that alanine at position +29 is
10 substituted with leucine, and leucine at position +104 is substituted with glutamic acid. The immunoglobulin portion of the mutant molecule can be mutated, so that the cysteines at positions +130, +136, and +139 are substituted with serine, and the proline at position +148 is substituted with serine. Alternatively, L104EA29LIg can have a CTLA4 portion encompassing alanine at position -1 through aspartic acid at position +124.

15 The invention provides L104EA29TIg (Figure 26) which is a mutant molecule including a CTLA4 portion encompassing methionine at position +1 through aspartic acid at position +124, a junction amino acid residue glutamine at position +125, and the immunoglobulin portion encompassing glutamic acid at position +126 through lysine at position +357. The portion
20 having the extracellular domain of CTLA4 is mutated so that alanine at position +29 is substituted with threonine, and leucine at position +104 is substituted with glutamic acid. The immunoglobulin portion of the mutant molecule can be mutated, so that the cysteines at positions +130, +136, and +139 are substituted with serine, and the proline at position +148 is substituted with serine. Alternatively, L104EA29TIg can have a CTLA4 portion encompassing alanine at
25 position -1 through aspartic acid at position +124.

The invention provides L104EA29WLIg (Figure 27) which is a mutant molecule including a CTLA4 portion encompassing methionine at position +1 through aspartic acid at position +124, a junction amino acid residue glutamine at position +125, and the immunoglobulin portion
30 encompassing glutamic acid at position +126 through lysine at position +357. The portion having the extracellular domain of CTLA4 is mutated so that alanine at position +29 is

substituted with tryptophan, and leucine at position +104 is substituted with glutamic acid. The immunoglobulin portion of the mutant molecule can be mutated, so that the cysteines at positions +130, +136, and +139 are substituted with serine, and the proline at position +148 is substituted with serine. Alternatively, L104EA29WIg can have a CTLA4 portion encompassing alanine at position -1 through aspartic acid at position +124.

The invention provides L104EG105FIg which is a mutant molecule including a CTLA4 portion encompassing methionine at position +1 through aspartic acid at position +124, a junction amino acid residue glutamine at position +125, and the immunoglobulin portion encompassing glutamic acid at position +126 through lysine at position +357. The portion having the extracellular domain of CTLA4 is mutated so that leucine at position +104 is substituted with glutamic acid, and glycine at position +105 is substituted with phenylalanine. Alternatively, L104EG105FIg can have a CTLA4 portion encompassing alanine at position -1 through aspartic acid at position +124.

15

The invention provides L104ES25RIg which is a mutant molecule including a CTLA4 portion encompassing methionine at position +1 through aspartic acid at position +124, a junction amino acid residue glutamine at position +125, and the immunoglobulin portion encompassing glutamic acid at position +126 through lysine at position +357. The portion having the extracellular domain of CTLA4 is mutated so that serine at position +25 is substituted with arginine, and leucine at position +104 is substituted with glutamic acid. Alternatively, L104ES25RIg can have a CTLA4 portion encompassing alanine at position -1 through aspartic acid at position +124.

20

The invention provides L104EA29YS25KIg which is a mutant molecule including a CTLA4 portion encompassing methionine at position +1 through aspartic acid at position +124, a junction amino acid residue glutamine at position +125, and the immunoglobulin portion encompassing glutamic acid at position +126 through lysine at position +357. The portion having the extracellular domain of CTLA4 is mutated so that serine at position +25 is substituted with lysine, alanine at position +29 is substituted with tyrosine, and leucine at position +104 is

30

substituted with glutamic acid. Alternatively, L104EA29YS25KIg can have a CTLA4 portion encompassing alanine at position -1 through aspartic acid at position +124.

5 The invention provides L104EA29YS25RIg which is a mutant molecule including a CTLA4 portion encompassing methionine at position +1 through aspartic acid at position +124, a junction amino acid residue glutamine at position +125, and the immunoglobulin portion encompassing glutamic acid at position +126 through lysine at position +357. The portion having the extracellular domain of CTLA4 is mutated so that serine at position +25 is substituted with arginine, alanine at position +29 is substituted with tyrosine, and leucine at position +104 is
10 substituted with glutamic acid. Alternatively, L104EA29YS25RIg can have a CTLA4 portion encompassing alanine at position -1 through aspartic acid at position +124.

The invention further provides nucleic acid molecules comprising nucleotide sequences encoding the amino acid sequences of the soluble CTLA4 mutant molecules of the invention. In
15 one embodiment, the nucleic acid molecule is a DNA (e.g., cDNA) or a hybrid thereof. Alternatively, the nucleic acid molecules are RNA or a hybrid thereof.

Additionally, the invention provides a vector which comprises the nucleotide sequences of the invention. A host vector system is also provided. The host vector system comprises the vector
20 of the invention in a suitable host cell. Examples of suitable host cells include but are not limited to prokaryotic and eukaryotic cells.

The invention further provides methods for producing a protein comprising growing the host vector system of the invention so as to produce the protein in the host and recovering the protein
25 so produced.

CTLA4Ig CODON-BASED MUTAGENESIS

In one embodiment, site-directed mutagenesis and a novel screening procedure were used to
30 identify several mutations in the extracellular domain of CTLA4 that improve binding avidity for CD86, while only marginally altering binding to CD80. In this embodiment, mutations

were carried out in residues in serine 25 to arginine 33, the C' strand (alanine 49 and threonine 51), the F strand (lysine 93, glutamic acid 95 and leucine 96), and in methionine 97 through tyrosine 102, tyrosine 103 through glycine 107 and in the G strand at positions glutamine 111, tyrosine 113 and isoleucine 115. These sites were chosen based on studies of chimeric CD28/CTLA4 fusion proteins (J. Exp. Med., 1994, 180:2049-2058), and on a model predicting which amino acid residue side chains would be solvent exposed, and a lack of amino acid residue identity or homology at certain positions between CD28 and CTLA4. Also, any residue which is spatially in close proximity (5 to 20 Angstrom Units) to the identified residues are considered part of the present invention.

To synthesize and screen soluble CTLA4 mutant molecules with altered affinities for CD86, a two-step strategy was adopted. The experiments entailed first generating a library of mutations at a specific codon of an extracellular portion of CTLA4 and then screening these by BIAcore analysis to identify mutants with altered reactivity to CD80 or CD86.

METHODS OF MAKING COMPOSITIONS OF THE INVENTION

Expression of CTLA4 mutant molecules in prokaryotic cells is preferred for some purposes. Prokaryotes most frequently are represented by various strains of bacteria. The bacteria may be a gram positive or a gram negative. Typically, gram-negative bacteria such as *E. coli* are preferred. Other microbial strains may also be used.

Sequences encoding CTLA4 mutant molecules can be inserted into a vector designed for expressing foreign sequences in prokaryotic cells such as *E. coli*. These vectors can include commonly used prokaryotic control sequences which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta-lactamase (penicillinase) and lactose (lac) promoter systems (Chang, et al., 1977 *Nature* 198:1056), the tryptophan (trp) promoter system (Goeddel, et al., 1980 *Nucleic Acids Res.* 8:4057) and the lambda derived P_L promoter and N-gene ribosome binding site (Shimatake, et al., 1981 *Nature* 292:128).

Such vectors will also include origins of replication and selectable markers, such as a beta-lactamase or neomycin phosphotransferase gene conferring resistance to antibiotics so that the vectors can replicate in bacteria and cells carrying the plasmids can be selected for when grown in the presence of ampicillin or kanamycin.

5 The expression plasmid can be introduced into prokaryotic cells via a variety of standard methods, including but not limited to CaCl_2 -shock (Cohen, 1972 *Proc. Natl. Acad. Sci. USA* 69:2110, and Sambrook et al. (eds.), "Molecular Cloning: A Laboratory Manual", 2nd Edition, Cold Spring Harbor Press, (1989)) and electroporation.

10 In accordance with the practice of the invention, eukaryotic cells are also suitable host cells. Examples of eukaryotic cells include any animal cell, whether primary or immortalized, yeast (e.g., *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Pichia pastoris*), and plant cells. Myeloma, COS and CHO cells are examples of animal cells that may be used as hosts.

15 Exemplary plant cells include tobacco (whole plants or tobacco callus), corn, soybean, and rice cells. Corn, soybean, and rice seeds are also acceptable.

Sequences encoding the CTLA4 mutant molecules can be inserted into a vector designed for expressing foreign sequences in a eukaryotic host. The regulatory elements of the vector can
20 vary according to the particular eukaryotic host.

Commonly used eukaryotic control sequences include promoters and control sequences compatible with mammalian cells such as, for example, CMV promoter (CDM8 vector) and avian sarcoma virus (ASV) (π LN vector). Other commonly used promoters include the early
25 and late promoters from Simian Virus 40 (SV40) (Fiers, et al., 1973 *Nature* 273:113), or other viral promoters such as those derived from polyoma, Adenovirus 2, and bovine papilloma virus. An inducible promoter, such as hMTII (Karin, et al., 1982 *Nature* 299:797-802) may also be used.

Vectors for expressing CTLA4 mutant molecules in eukaryotes may also carry sequences called enhancer regions. These are important in optimizing gene expression and are found either upstream or downstream of the promoter region.

- 5 Sequences encoding CTLA4 mutant molecules can integrate into the genome of the eukaryotic host cell and replicate as the host genome replicates. Alternatively, the vector carrying CTLA4 mutant molecules can contain origins of replication allowing for extrachromosomal replication.

For expressing the sequences in *Saccharomyces cerevisiae*, the origin of replication from the
10 endogenous yeast plasmid, the 2 μ circle could be used. (Broach, 1983 *Meth. Enz.* 101:307). Alternatively, sequences from the yeast genome capable of promoting autonomous replication could be used (see, for example, Stinchcomb et al., 1979 *Nature* 282:39); Tschemper et al., 1980 *Gene* 10:157; and Clarke et al., 1983 *Meth. Enz.* 101:300).

- 15 Transcriptional control sequences for yeast vectors include promoters for the synthesis of glycolytic enzymes (Hess et al., 1968 *J. Adv. Enzyme Reg.* 7:149; Holland et al., 1978 *Biochemistry* 17:4900). Additional promoters known in the art include the CMV promoter provided in the CDM8 vector (Toyama and Okayama, 1990 *FEBS* 268:217-221); the promoter for 3-phosphoglycerate kinase (Hitzeman et al., 1980 *J. Biol. Chem.* 255:2073), and those for
20 other glycolytic enzymes.

Other promoters are inducible because they can be regulated by environmental stimuli or the growth medium of the cells. These inducible promoters include those from the genes for heat shock proteins, alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, enzymes
25 associated with nitrogen catabolism, and enzymes responsible for maltose and galactose utilization.

- Regulatory sequences may also be placed at the 3' end of the coding sequences. These sequences may act to stabilize messenger RNA. Such terminators are found in the 3' untranslated region
30 following the coding sequences in several yeast-derived and mammalian genes.

Exemplary vectors for plants and plant cells include but are not limited to *Agrobacterium* T₁ plasmids, cauliflower mosaic virus (CaMV), tomato golden mosaic virus (TGMV).

General aspects of mammalian cell host system transformations have been described by Axel (U.S. Patent No. 4,399,216 issued Aug. 16, 1983). Mammalian cells can be transformed by methods including but not limited to, transfection in the presence of calcium phosphate, microinjection, electroporation, or via transduction with viral vectors.

Methods for introducing foreign DNA sequences into plant and yeast genomes include (1) mechanical methods, such as microinjection of DNA into single cells or protoplasts, vortexing cells with glass beads in the presence of DNA, or shooting DNA-coated tungsten or gold spheres into cells or protoplasts; (2) introducing DNA by making protoplasts permeable to macromolecules through polyethylene glycol treatment or subjection to high voltage electrical pulses (electroporation); or (3) the use of liposomes (containing cDNA) which fuse to protoplasts.

Expression of CTLA4 mutant molecules can be detected by methods known in the art. For example, the mutant molecules can be detected by Coomassie staining SDS-PAGE gels and immunoblotting using antibodies that bind CTLA4. Protein recovery can be performed using standard protein purification means, e.g., affinity chromatography or ion-exchange chromatography, to yield substantially pure product (R. Scopes in: "Protein Purification, Principles and Practice", Third Edition, Springer-Verlag 1994).

The soluble CTLA4 mutant molecule can be isolated by molecular or chemical synthesis methods. The molecular methods may include the following steps: introducing a suitable host cell with a nucleic acid molecule that expresses and encodes the soluble CTLA4 mutant molecule; culturing the host cell so introduced under conditions that permit the host cell to express the mutant molecules; and isolating the expressed mutant molecules. The signal peptide portion of the mutant molecule permits the expressed protein molecules to be secreted by the host cell. The secreted mutant molecules can undergo post-translational modification, involving cleavage of the signal peptide to produce a mature protein having the CTLA4 and the

immunoglobulin portions. The cleavage may occur after the alanine at position -1, resulting in a mature mutant molecule having methionine at position +1 as the first amino acid (Figure 22). Alternatively, the cleavage may occur after the methionine at position -2, resulting in a mature mutant molecule having alanine at position -1 as the first amino acid.

Making Monoclonal Antibodies of the invention

Monoclonal antibodies reactive with *CTLA4* mutant molecules, may be produced by hybridomas prepared using known procedures, such as those introduced by Kohler and Milstein (Kohler and Milstein, Nature, 256:495-97 (1975)), and modifications thereof, to regulate cellular interactions.

These techniques involve the use of an animal which is primed to produce a particular antibody. The animal can be primed by injection of an immunogen (e.g. a soluble *CTLA4* mutant molecule) to elicit the desired immune response, i.e. production of antibodies from the primed animal. Lymphocytes derived from the lymph nodes, spleens or peripheral blood of primed, diseased animals can be used to search for a particular antibody. The lymphocyte chromosomes encoding desired immunoglobulins are immortalized by fusing the lymphocytes with myeloma cells, generally in the presence of a fusing agent such as polyethylene glycol (PEG). Any of a number of myeloma cell lines may be used as a fusion partner according to standard techniques; for example, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653, Sp2/0-Ag14, or HL1-653 myeloma lines. These myeloma lines are available from the ATCC, Manassas, Maryland.

The resulting cells, which include the desired hybridomas, are then grown in a selective medium such as HAT medium, in which unfused parental myeloma or lymphocyte cells eventually die. Only the hybridoma cells survive and can be grown under limiting dilution conditions to obtain isolated clones. The supernatants of the hybridomas are screened for the presence of antibodies of the desired specificity, e.g. by immunoassay techniques using the *CTLA4* mutant molecule that has been used for immunization. Positive clones can then be subcloned under limiting dilution conditions, and the monoclonal antibody produced can be isolated.

Various conventional methods can be used for isolation and purification of the monoclonal antibodies so as to obtain them free from other proteins and contaminants. Commonly used methods for purifying monoclonal antibodies include ammonium sulfate precipitation, ion exchange chromatography, and affinity chromatography (Zola et al., in Monoclonal Hybridoma Antibodies: Techniques and Applications, Hurell (ed.) pp. 51-52 (CRC Press, 1982)). Hybridomas produced according to these methods can be propagated in vitro or in vivo (in ascites fluid) using techniques known in the art (Fink et al., Prog. Clin. Pathol., 9:121-33 (1984)).

Generally, the individual cell line may be propagated in vitro, for example, in laboratory culture vessels, and the culture medium containing high concentrations of a single specific monoclonal antibody can be harvested by decantation, filtration, or centrifugation.

In addition, fragments of these antibodies containing the active binding region reactive with the extracellular domain of *CTLA4* mutant molecule, such as Fab, F(ab')₂ and Fv fragments may be produced. Such fragments can be produced using techniques well established in the art (e.g. Rousseaux et al., in Methods Enzymol., 121:663-69, Academic Press (1986)).

METHODS FOR USING THE COMPOSITIONS OF THE INVENTION

Additionally, the invention provides methods for regulating functional CTLA4- and CD28-positive T cell interactions with CD80- and/or CD86-positive cells. The methods comprise contacting the CD80- and/or CD86-positive cells with a soluble CTLA4 mutant molecule of the invention thereby regulating functional CTLA4 and/or CD28 T cell interactions with CD80- and/or CD86-positive cells. In one embodiment of the invention, the soluble CTLA4 mutant molecule is a fusion protein that contains at least a portion of the extracellular domain of mutant CTLA4. In another embodiment, the soluble CTLA4 mutant molecule comprises: a first amino acid sequence including the extracellular domain of CTLA4 from methionine at position +1 to aspartic acid at position +124, including at least one mutation; and a second amino acid sequence including the hinge, CH2, and CH3 regions of the human immunoglobulin gamma 1 molecule (Figure 3 or 22).

The present invention further provides a method for treating immune system diseases (also referred to herein as immunoproliferative diseases) mediated by CD28- and/or CTLA4-positive cell interactions with CD80/CD86-positive cells. In one embodiment, T cell interactions are inhibited. This method comprises administering to a subject the soluble CTLA4 mutant molecules of the invention to regulate T cell interactions with the CD80- and/or CD86-positive cells.

The present invention also provides method for inhibiting graft versus host disease in a subject. This method comprises administering to the subject a soluble CTLA4 mutant molecule of the invention.

The invention encompasses the use of the soluble CTLA4 mutant molecules together with other immunosuppressants, e.g., cyclosporin (Mathiesen, in: "Prolonged Survival and Vascularization of Xenografted Human Glioblastoma Cells in the Central Nervous System of Cyclosporin A-Treated Rats" 1989 *Cancer Lett.*, 44:151-156), prednisone, azathioprine, and methotrexate (R. Handschumacher "Chapter 53: Drugs Used for Immunosuppression" pages 1264-1276). Other immunosuppressants are possible.

CTLA4 mutant molecules may be used to react with CD80 or CD86 positive cells, such as B cells, to regulate immune responses mediated by T cell interactions with the B7 antigen positive cells or in vitro for leukocyte typing so as to define B cell maturational stages and/or B cell associated diseases (Yokochi et al. *J. Immuno.* 128(2):823. Surface immunostaining of leukocytes is accomplished by immunofluorescent technology or immunoenzymatic methods but other means of detection are possible.

Soluble CTLA4 mutant molecules may also be used to react with B7 positive cells, including B cells, to regulate immune responses mediated by T cell dependent B cell responses.

The B7 antigen expressed on activated B cells and cells of other lineages, and the CD28 receptor expressed on T cells, can directly bind to each other, and this interaction can mediate cell-cell interaction. Such interactions directly trigger the CD28/B7 activation pathway in T cells, leading to cytokine production, T cell proliferation, and B cell differentiation into immunoglobulin

producing cells. The activation of B cells that occurs, can cause increased expression of B7 antigen and further CD28 stimulation, leading to a state of chronic inflammation such as in autoimmune diseases, allograft rejection, graft versus host disease or chronic allergic reactions. Blocking or inhibiting this reaction may be effective in preventing T cell cytokine production and T/B cell proliferation and thus preventing or reversing inflammatory reactions.

Soluble CTLA4 mutant molecule can be a potent inhibitor of in vitro lymphocyte functions requiring T and B cell interaction. This indicates the importance of interactions between the B7 antigen and its counter-receptors, CTLA4 and/or CD28.

The soluble CTLA4 mutant molecules are expected to exhibit inhibitory properties in vivo. Under conditions where T cell/B cell interactions are occurring as a result of contact between T cells and B cells, binding of introduced CTLA4 mutant molecules to react with B7 antigen positive cells, for example B cells, may interfere, i.e. inhibit, the T cell/B cell interactions resulting in regulation of immune responses. Because of this exclusively inhibitory effect, CTLA4 mutant molecule is expected to be useful in vivo as an inhibitor of T cell activity, over non-specific inhibitors such as cyclosporine and glucosteroids.

In one embodiment, the CTLA4 mutant molecules may be introduced in a suitable pharmaceutical carrier in vivo, i.e. administered into a subject, e.g., a human subject, for treatment of pathological conditions such as immune system diseases or cancer.

Introduction of CTLA4 mutant molecules in vivo is expected to result in interference with T cell interactions with other cells, such as B cells, as a result of binding of the ligand to B7 positive cells. The prevention of normal T cell interactions may result in decreased T cell activity, for example, decreased T cell proliferation or alter cytokine production.

Under some circumstances, as noted above, the effect of administration of the CTLA4 mutant molecules in vivo is inhibitory, resulting from blocking by CTLA4 mutant molecules and CD28 triggering resulting from T cell/B cell contact. For example, the CTLA4 mutant molecules may block T cell proliferation. Introduction of the CTLA4 mutant molecules in vivo will thus

produce effects on both T and B cell-mediated immune responses. The mutant molecules may also be administered to a subject in combination with the introduction of cytokines or other therapeutic reagents.

- 5 In an additional embodiment of the invention, other reagents, including derivatives reactive with the CTLA4 mutant molecules are used to regulate T cell interactions. For example, antibodies, and/or antibody fragments reactive with the CTLA4 mutant molecules can be screened to identify those capable of inhibiting the binding of the CTLA4 mutant molecules to CD80 or CD86. The antibodies or antibody fragments such as Fab or F(ab')₂ fragments, may then be used
10 to react with the T cells, for example, to inhibit T cell proliferation.

In another embodiment, the CTLA4 mutant molecules may be used to identify additional compounds capable of regulating the interaction between CTLA4 and CD80 or CD86. Such compounds may include small naturally occurring molecules that can be used to react with B
15 cells and/or T cells. For example, fermentation broths may be tested for the ability to inhibit CTLA4/B7 interactions.

The CD28-mediated T cell proliferation pathway is cyclosporine-resistant, in contrast to proliferation driven by the CD3/Ti cell receptor complex (June et al., 1987, supra). Cyclosporine is relatively ineffective as a treatment for GVH disease (Storb, Blood 68:119-125 (1986)). GVH
20 disease is thought to be mediated by T lymphocytes which express CD28 antigen (Storb and Thomas, Immunol. Rev. 88:215-238 (1985)). Thus, the *CTLA4* mutant molecules may be useful alone, or in combination with immunosuppressants such as cyclosporine, for blocking T cell proliferation in GVH disease.

- 25 Regulation of *CTLA4*-positive T cell interactions with B7 positive cells, including B cells, by the methods of the invention may thus be used to treat pathological conditions such as autoimmunity, transplantation, infectious diseases and neoplasia.

The B7-binding molecules described herein may be in a variety of dosage forms which include,
30 but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories,

polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

5 The most effective mode of administration and dosage regimen for the molecules of the present invention depends upon the severity and course of the disease, the subject's health and response to treatment and the judgment of the treating physician. Accordingly, the dosages of the molecules should be titrated to the individual subject.

10 The interrelationship of dosages for animals of various sizes and species and humans based on mg/m^2 of surface area is described by Freireich, E.J., et al. (Quantitative Comparison of Toxicity of Anticancer Agents in Mouse, Rat, Hamster, Dog, Monkey and Man. Cancer Chemother, Rep., 50, No.4, 219-244, May 1966).

15 Adjustments in the dosage regimen may be made to optimize the growth inhibiting response. Doses may be divided and administered on a daily basis or the dose may be reduced proportionally depending upon the situation. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the specific therapeutic situation.

20 In accordance with the practice of the invention an effective amount for treating a subject may be between about 0.1 and about 10mg/kg body weight of subject. Also, the effective amount may be an amount between about 1 and about 10 mg/kg body weight of subject.

25 The CTLA4 mutant molecules of the invention also have in vitro clinical application. They may be useful in the enumeration of B7 positive cells in the diagnosis or prognosis of some conditions of immunodeficiency, the phenotyping of leukemias and lymphomas, and the monitoring of immunological change following organ transplantation.

ADVANTAGES OF THE PRESENT INVENTION

5 The discovery of additional CTLA4 mutants are important because they can be important immunosuppressive agents or tools to combat disease. Additionally, soluble CTLA4 mutant molecules having a higher avidity for CD80- or CD86- positive cells compared to wild type CTLA4 have an advantage over wildtype CTLA4 molecules because they are expected to block the priming of antigen specific activated cells with higher efficiency than wild type.

10 Further, production costs for CTLA4Ig are very high. The high avidity mutant CTLA4Ig molecules having higher potent immunosuppressive properties could be used in the clinic at considerably lower doses than non-mutated CTLA4Ig to achieve similar levels of immunosuppression. Soluble CTLA4 mutant molecules, e.g., L104EA29YIg, could be very
15 cost effective.

The following example is presented to illustrate the present invention and to assist one of ordinary skill in making and using the same. This example is not intended in any way to otherwise limit the scope of the invention.

EXAMPLE 1

20 The following provides a description of the methods used to generate a nucleotide sequence encoding the CTLA4Ig fusion protein.

Preparation of CTLA4Ig Fusion Protein

25 A genetic construct encoding CTLA4Ig between the extracellular domain of CTLA4 and an IgGgamma1 domain was constructed in a manner similar to that described above for the CD28Ig construct. The extracellular domain of the CTLA4 gene was cloned by PCR using synthetic
30 oligonucleotides corresponding to the published sequence (Dariavach et al., Eur. Journ. Immunol. 18:1901-1905 (1988)).

Because a signal peptide for CTLA4 was not identified in the CTLA4 gene, the N-terminus of the predicted sequence of CTLA4 was fused to the signal peptide of oncostatin M (Malik et al., Mol. and Cell. Biol. 9:2847 (1989)) in two steps using overlapping oligonucleotides. For the

5 first step, the oligonucleotide, CTCAGTCTGGTCCTTGCACTCCTG
TTTCCAAGCATGGCGAGCATGGCAATGCACGTGGCCCAGCC (which encoded the C
terminal 15 amino acids from the oncostatin M signal peptide fused to the N terminal 7 amino
acids of CTLA4) was used as forward primer, and
10 TTTGGGCTCCTGATCAGAATCTGGGCACGGTTG (encoding amino acid residues 119-125
of the amino acid sequence encoding CTLA4 receptor and containing a Bcl I restriction enzyme
site) as reverse primer. The template for this step was cDNA synthesized from 1 micro g of total
RNA from H38 cells (an HTLV II infected T cell leukemic cell line provided by Drs. Salahudin
and Gallo, NCI, Bethesda, MD). A portion of the PCR product from the first step was
15 reamplified, using an overlapping forward primer, encoding the N terminal portion of the
oncostatin M signal peptide and containing a Hind III restriction endonuclease site,
CTAGCCACTGAAGCTTCACCAATGGGTGTACTGCTCACACAGAGGACGCTGCTCAG
TCTGGTCCTTGCACTC and the same reverse primer. The product of the PCR reaction was
digested with Hind III and Bcl I and ligated together with a Bcl I/Xba I cleaved cDNA fragment
encoding the amino acid sequences corresponding to the hinge, CH2 and CH3 regions of IgC \square 1
20 into the Hind III/Xba I cleaved expression vector, CDM8 or Hind III/Xba I cleaved expression
vector pLN.

A schematic map of the resulting CTLA4Ig fusion construct is shown in Figure 1. Sequences
displayed in this figure show the junctions between CTLA4 (upper case letters, unshaded
25 regions) and the signal peptide, SP, of oncostatin M (dark shaded regions), and the hinge, H, of
IgC(gamma)1 (stippled regions). The amino acid in parentheses was introduced during
construction. Asterisks (*) indicate cysteine to serine mutations introduced in the IgC \square hinge
region. The immunoglobulin superfamily V-like domain present in CTLA4 is indicated, as are
the CH2 and CH3 domains of IgC(gamma)1.

30

Expression plasmids, CDM8, containing CTLA4Ig were then transfected into COS cells using DEAE/dextran transfection by modification (Linsley et al., 1991, supra) of the protocol described by Seed and Aruffo, 1987, supra.

- 5 Expression plasmid constructs (pi LN or CDM8) containing cDNA encoding the amino acid sequence of CTLA4Ig, was transfected by lipofection using standard procedures into dhfr⁻ CHO lines to obtain novel cell lines stably expressing CTLA4Ig.

10 DNA encoding the amino acid sequence corresponding to CTLA4Ig has been deposited with the ATCC under the Budapest Treaty on May 31, 1991, and has been accorded ATCC accession number 68629.

15 A preferred stable transfectant, expressing CTLA4Ig, designated Chinese Hamster Ovary Cell Line, CTLA4Ig-24, was made by screening B7 positive CHO cell lines for B7 binding activity in the medium using immunostaining. Transfectants were maintained in DMEM supplemented with 10% fetal bovine serum (FBS), 0.2 mM proline and 1 micro M methotrexate.

The CTLA4Ig-24 CHO cell line has been deposited with the ATCC under the Budapest Treaty on May 31, 1991 and has been accorded accession number ATCC 10762.

20

CTLA4Ig was purified by protein A chromatography from serum-free conditioned supernatants (Figure 2). Concentrations of CTLA4Ig were determined assuming an extinction coefficient at 280 nm of 1.6 (experimentally determined by amino acid analysis of a solution of known absorbance). Molecular weight standards (lanes 1 and 3, Figure 2) and samples (1 micro grams) of CTLA4Ig (lanes 2 and 4) were subjected to SDS-PAGE (4-12% acrylamide gradient) under 25 non-reducing conditions (-βME, lanes 1 and 2) or reducing conditions (+ beta ME, lanes 3 and 4). Proteins were visualized by staining with Coomassie Brilliant Blue.

30 Under non-reducing conditions, CTLA4Ig migrated as a M_r approximately 100,000 species, and under reducing conditions, as a M_r approximately 50,000 species (Figure 2). Because the IgC

(gamma) hinge disulfides were eliminated during construction, CTLA4Ig, like CD28Ig, is a dimer presumably joined through a native disulfide linkage.

EXAMPLE 2

5 The following provides a description of the methods used to generate the nucleotide sequence encoding the CTLA4 receptor.

CTLA4 Receptor

10 To reconstruct DNA encoding the amino acid sequence corresponding to the full length human CTLA4 gene, cDNA encoding amino acids corresponding to a fragment of the transmembrane and cytoplasmic domains of CTLA4 was cloned by PCR and then joined with cDNA encoding amino acids corresponding to a fragment from CTLA4Ig that corresponded to the oncostatin M
15 signal peptide fused to the N-terminus of CTLA4. Procedures for PCR, and cell culture and transfections were as described above in Example 1 using COS cells and DEAE-dextran transfection.

20 Because the expression of CTLA4 receptor protein in human lymphoid cells has not been previously reported, it was necessary to locate a source of CTLA4 mRNA. cDNA was reverse transcribed from the total cellular RNA of H38 cells, as noted above, was used for cloning by PCR.

For this purpose, the oligonucleotide,
GCAATGCACGTGGCCCAGCCTGCTGTGGTAGTG

25 (encoding the first 11 amino acids in the predicted coding sequence) was used as a forward primer, and TGATGTAACATGTCTAGATCAATTGATGGGAATAAAATAAGGCTG (homologous to the last 8 amino acids in CTLA4 and containing a Xba I site) as reverse primer.

The template again was a cDNA synthesized from 1 micro gram RNA from H38 cells. Products of the PCR reaction were cleaved with the restriction endonucleases Nco I and Xba I and the resulting 316 bp product was gel purified. A 340 bp Hind III/Nco I fragment from the CTLA4Ig
30 fusion described above was also gel-purified, and both restriction fragments were ligated into Hind III/Xba I cleaved CDM8 to form OMCTLA.

The resulting construct corresponded to full length CTLA4 and the oncostatin M signal peptide. The nucleotide sequence of the construct is shown in Figure 3 and was designated OMCTLA4. The sequence for CTLA4 shown in Figure 3 differs from the predicted human CTLA4 DNA sequence (Dariavach et al., supra) by a base change such that the previously reported alanine at amino acid position 110 of the amino acid sequence shown, encodes a threonine. This threonine is part of a newly identified N-linked glycosylation site that may be important for successful expression of the fusion protein.

10 Ligation products were transformed into MC1061/p3 *E. coli* cells and colonies were screened for the appropriate plasmids. Sequences of the resulting constructs were confirmed by DNA sequence analysis.

EXAMPLE 3

15 The following provides a description of the methods used to generate the nucleotide sequences encoding the B7Ig and CD28Ig fusion proteins.

Preparation of B7Ig and CD28Ig Fusion Proteins

20 Receptor-immunoglobulin C gamma (IgC γ) fusion proteins B7Ig and CD28Ig were prepared as described by Linsley et al., in *J. Exp. Med.* 173:721-730 (1991), incorporated by reference herein. Briefly, DNA encoding amino acid sequences corresponding to the respective receptor protein (e.g. B7) was joined to DNA encoding amino acid sequences corresponding to the hinge, CH2 and CH3 regions of human IgC γ 1. This was accomplished as follows.

25 Polymerase Chain Reaction (PCR). For PCR, DNA fragments were amplified using primer pairs as described below for each fusion protein. PCR reactions (0.1 ml final volume) were run in *Taq* polymerase buffer (Stratagene, La Jolla, CA), containing 20 micro moles each of dNTP; 50-100 pmoles of the indicated primers; template (1 ng plasmid or cDNA synthesized from \leq 1 micro gram total RNA using random hexamer primer, as described by Kawasaki in PCR Protocols,

Academic Press, pp. 21-27 (1990), incorporated by reference herein); and Taq polymerase (Stratagene). Reactions were run on a thermocycler (Perkin Elmer Corp., Norwalk, CT) for 16-30 cycles (a typical cycle consisted of steps of 1 min at 94 degrees C, 1-2 min at 50 degrees C and 1-3 min at 72 degrees C).

5 Plasmid Construction. Expression plasmids containing cDNA encoding CD28, (as described by Aruffo and Seed, Proc. Natl. Acad. Sci. USA 84:8573 (1987)), were provided by Drs. Aruffo and Seed (Mass General Hospital, Boston, MA). Plasmids containing cDNA encoding CD5, (as described by Aruffo, Cell 61:1303 (1990)), were provided by Dr. Aruffo. Plasmids containing
10 cDNA encoding B7, (as described by Freeman et al., J. Immunol. 143:2714 (1989)), were provided by Dr. Freeman (Dana Farber Cancer Institute, Boston, MA). For initial attempts at expression of soluble forms of CD28 and B7, constructs were made (OMCD28 and OMB7) as described by Linsley et al., J. Exp. Med., supra, in which stop codons were introduced upstream of the transmembrane domains and the native signal peptides were replaced with the signal
15 peptide from oncostatin M (Malik et al., Mol. Cell Biol. 9:2847 (1989)). These were made using synthetic oligonucleotides for reconstruction (OMCD28) or as primers (OMB7) for PCR. OMCD28, is a CD28 cDNA modified for more efficient expression by replacing the signal peptide with the analogous region from oncostatin M. CD28Ig and B7Ig fusion constructs were made in two parts. The 5' portions were made using OMCD28 and OMB7 as templates and the
20 oligonucleotide, CTAGCCACTGAAGCTTCACCATGGGTGTACTGCTCACAC (encoding the amino acid sequence corresponding to the oncostatin M signal peptide) as a forward primer, and either TGGCATGGGCTCCTGATCAGGCTTAGAAGGTCCGGGAAA or, TTTGGGCTCCTGATCAGGAAAATGCTCTTGCTTGGTTGT as reverse primers, respectively. Products of the PCR reactions were cleaved with restriction endonucleases (Hind
25 III and BclI) as sites introduced in the PCR primers and gel purified.

The 3' portion of the fusion constructs corresponding to human IgC α 1 sequences was made by a coupled reverse transcriptase (from Avian myeloblastosis virus; Life Sciences Associates, Bayport, NY)-PCR reaction using RNA from a myeloma cell line producing human-mouse
30 chimeric mAb L6 (provided by Dr. P. Fell and M. Gayle, Bristol-Myers Squibb Company, Pharmaceutical Research Institute, Seattle, WA) as template. The oligonucleotide,

AAGCAAGAGCATTTTCCTGATCAGGAGCCCAAATCTTCTGACAAAACACACATC
CCCACCGTCCCCAGCACCTGAACTCCTG was used as forward primer, and
CTTCGACCAGTCTAGAAGCATCCTCGTGCGACCGCGAGAGC

as reverse primer. Reaction products were cleaved with BclI and XbaI and gel purified. Final
5 constructs were assembled by ligating HindIII/BclI cleaved fragments containing CD28 or B7
sequences together with BclI/XbaI cleaved fragment containing IgC α 1 sequences into
HindIII/XbaI cleaved CDM8. Ligation products were transformed into MC1061/p3 *E. coli* cells
and colonies were screened for the appropriate plasmids. Sequences of the resulting constructs
were confirmed by DNA sequencing.

10 The construct encoding B7 contained DNA encoding amino acids corresponding to amino acid
residues from approximately position 1 to approximately position 215 of the extracellular
domain of B7. The construct encoding CD28 contained DNA encoding amino acids
corresponding to amino acid residues from approximately position 1 to approximately position
15 134 of the extracellular domain of CD28.

CD5Ig was constructed in identical fashion, using

CATTGCACAGTCAAGCTTCCATGCCCATGGGTTCTCTGGCCACCTTG as forward
primer and ATCCACAGTGCAGTGATCATTTGGATCCTGGCATGTGAC as reverse primer.

20 The PCR product was restriction endonuclease digested and ligated with the IgC α 1 fragment as
described above. The resulting construct (CD5Ig) encoded a mature protein having an amino
acid sequence containing amino acid residues from position 1 to position 347 of the sequence
corresponding to CD5, two amino acids introduced by the construction procedure (amino acids
DQ), followed by DNA encoding amino acids corresponding to the IgC γ 1 hinge region.

25 Cell Culture and Transfections. COS (monkey kidney cells) were transfected with expression
plasmids expressing CD28 and B7 using a modification of the protocol of Seed and Aruffo
(*Proc. Natl. Acad. Sci.* 84:3365 (1987)), incorporated by reference herein. Cells were seeded at
10⁶ per 10 cm diameter culture dish 18-24 h before transfection. Plasmid DNA was added
30 (approximately 15 micro grams/dish) in a volume of 5 mls of serum-free DMEM containing 0.1
mM chloroquine and 600 micro grams/ml DEAE Dextran, and cells were incubated for 3-3.5 h

at 37 degrees C. Transfected cells were then briefly treated (approximately 2 min) with 10% dimethyl sulfoxide in PBS and incubated at 37 degrees C for 16-24 h in DMEM containing 10% FCS. At 24 h after transfection, culture medium was removed and replaced with serum-free DMEM (6 ml/dish). Incubation was continued for 3 days at 37 degrees C, at which time the spent medium was collected and fresh serum-free medium was added. After an additional 3 days at 37 degrees C, the spent medium was again collected and cells were discarded.

CHO cells expressing CD28, CD5 or B7 were isolated as described by Linsley et al., (1991) supra, as follows: Briefly, stable transfectants expressing CD28, CD5, or B7, were isolated following cotransfection of dihydrofolate reductase-deficient Chinese hamster ovary (dhfr CHO) cells with a mixture of the appropriate expression plasmid and the selectable marker, pSV2dhfr (Linsley et al., Proc. Natl. Acad. Sci. USA 87:5031 (1990)), incorporated by reference herein. Transfectants were then grown in increasing concentrations of methotrexate to a final level of 1 micro M and were maintained in DMEM supplemented with 10% fetal bovine serum (FBS), 0.2 mM proline and 1 micro M methotrexate. CHO lines expressing high levels of CD28 (CD28 CHO) or B7 (B7⁺ CHO) were isolated by multiple rounds of fluorescence-activated cell sorting (FACS^R) following indirect immunostaining with mAbs 9.3 or BB-1. Amplified CHO cells negative for surface expression of CD28 or B7 (dhfr⁺ CHO) were also isolated by FACS^R from CD28-transfected populations.

Immunostaining and FACS^R Analysis. Transfected CHO or COS cells or activated T cells were analyzed by indirect immunostaining. Before staining, CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with murine mAbs 9.3 (Hansen et al., Immunogenetics 10:247 (1980)) or BB-1 (Yokochi et al., J. Immunol. 128:823 (1981)), or with Ig fusion proteins (all at 10 micro grams/ml in DMEM containing 10% FCS) for 1-2 h at 4 °C. Cells were then washed, and incubated for an additional 0.5-2h at 4 °C with a FITC-conjugated second step reagent (goat anti-mouse Ig serum for murine mAbs, or goat anti-human Ig G serum for fusion proteins (Tago, Inc., Burlingame, CA)). Fluorescence was analyzed on a FACS IV^R cell sorter (Becton Dickinson and CO., Mountain View, CA) equipped with a four decade logarithmic amplifier.

Purification of Ig Fusion Proteins. The first, second and third collections of spent serum-free culture media from transfected COS cells were used as sources for the purification of Ig fusion proteins. After removal of cellular debris by low speed centrifugation, medium was applied to a column (approximately 200-400 ml medium/ml packed bed volume) of immobilized protein A (Repligen Corp., Cambridge, MA) equilibrated with 0.05 M sodium citrate, pH 8.0. After application of the medium, the column was washed with 1 M potassium phosphate, pH 8, and bound protein was eluted with 0.05 M sodium citrate, pH 3. Fractions were collected and immediately neutralized by addition of 1/10 volume of 2 M Tris, pH 8. Fractions containing the peak of A₂₈₀ absorbing material were pooled and dialyzed against PBS before use. Extinction coefficients of 2.4 and 2.8 ml/mg for CD28Ig and B7Ig, respectively, were determined by amino acid analysis of solutions of known absorbance. The recovery of purified CD28Ig and B7Ig binding activities was nearly quantitative as judged by FACS^R analysis after indirect fluorescent staining of B7⁺ and CD28⁺ CHO cells.

EXAMPLE 4

The following provides a description of the methods used to characterize CTLA4Ig.

Characterization of CTLA4Ig

To characterize the CTLA4Ig constructs, several isolates, CD28Ig, B7Ig, and CD5Ig, were prepared as described above and were transfected into COS cells as described in Examples 2 and 3, and were tested by FACS^R analysis for binding of B7Ig. In addition to the above-mentioned constructs, CDM8 plasmids containing cDNAs encoding CD7 as described by Aruffo and Seed, (EMBO Jour. 6:3313-3316 (1987)), incorporated by reference herein, were also used.

mAbs. Murine monoclonal antibodies (mAbs) 9.3 (anti-CD28) and G19-4 (anti-CD3), G3-7 (anti-CD7), BB-1 (anti-B7 antigen) and rat mAb 187.1 (anti-mouse kappa chain) have been described previously (Ledbetter et al., Proc. Natl. Acad. Sci. 84:1384-1388 (1987); Ledbetter et al., Blood 75:1531 (1990); Yokochi et al., supra) and were purified from ascites before use. The

hybridoma producing mAb OKT8 was obtained from the ATCC, Rockville, MD, and the mAb was also purified from ascites before use. mAb 4G9 (anti-CD19) was provided by Dr. E. Engleman, Stanford University, Palo Alto, CA). Purified human-mouse chimeric mAb L6 (having human C α 1 Fc portion) was a gift of Dr. P. Fell and M. Gayle (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA).

Immunostaining and FACS^R Analysis. Prior to staining, COS or CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with mAbs or Ig fusion proteins at 10 micro grams/ml in DMEM containing 10% FBS for 1-2 hr at 4 degrees C. Cells were then washed, and incubated for an additional 0.5-2 hrs at 4 degrees C with FITC-conjugated goat anti-mouse immunoglobulin or with FITC-conjugated goat anti-human Ig C α serum (both from Tago, Burlingame, CA). When binding of both mAbs and Ig fusion proteins were measured in the same experiment, FITC-conjugated anti-mouse and anti-human second step reagents were mixed together before use. Fluorescence on a total of 10,000 cells was then analyzed by FACS^R.

Peripheral Blood Lymphocyte Separation and Stimulation. Peripheral blood lymphocytes (PBLs) were isolated by centrifugation through Lymphocyte Separation Medium (Litton Bionetics, Kensington, MD). Alloreactive T cells were isolated by stimulation of PBL in a primary mixed lymphocyte reaction (MLR). PBL were cultured at 10^6 /ml irradiated (5000 rad) T51 LCL. EBV-transformed lymphoblastoid cell lines (LCL), PM (Bristol-Myers Squibb Co.) and T51 (Bristol-Myers Squibb Co.) were maintained in RPMI supplemented with 10% FBS. After 6 days, alloreactive "blasts" cells were cryopreserved. Secondary MLR were conducted by culturing thawed alloreactive blasts together with fresh irradiated T51 LCL in the presence and absence of mAbs and Ig fusion proteins. Cells were cultured in 96 well flat bottom plates (4×10^4 alloreactive blasts and 1×10^4 irradiated T51 LCL cells/well, in a volume of 0.2 ml) in RPMI containing 10% FBS. Cellular proliferation of quadruplicate cultures was measured by uptake of [³H]-thymidine during the last 6 hours of a 2-3 day culture.

PHA-activated T cells were prepared by culturing PBLs with 1 micro g/ml PHA (Wellcome, Charlotte, NC) for five days, and one day in medium lacking PHA. Viable cells were collected

by sedimentation through Lymphocyte Separation Medium before use. Cells were stimulated with mAbs or transfected CHO cells for 4-6 hr at 37 degrees C, collected by centrifugation and used to prepare RNA.

5 CD4⁺ T cells were isolated from PBLs by separating PBLs from healthy donors into T and non-T cells using sheep erythrocyte rosetting technique and further separating T cells by panning into CD4⁺ cells as described by Damle et al., J. Immunol. 139:1501 (1987), incorporated by reference herein.

10 B cells were also purified from peripheral blood by panning as described by Wysocki and Sato, Proc. Natl. Acad. Sci. 75:2844 (1978), incorporated by reference herein, using anti-CD19 mAb 4G9. To measure T_H-induced Ig production, 10⁶ CD4⁺ T cells were mixed with 10⁶ CD19⁺ B cells in 1 ml of RPMI containing 10% FBS. Following culture for 6 days at 37 degrees C, production of human IgM was measured in the culture supernatants using solid phase ELISA as
15 described by Volkman et al., Proc. Natl. Acad. Sci. USA 78:2528 (1981), incorporated by reference herein.

Briefly, 96-well flat bottom microtiter ELISA plates (Corning, Corning, NY) were coated with 200 micro liter/well of sodium carbonate buffer (pH 9.6) containing 10 micro grams/ml of
20 affinity-purified goat anti-human IgG or IgM antibody (Tago, Burlingame, CA), incubated overnight at 4°C, and then washed with PBS and wells were further blocked with 2% BSA in PBS (BSA-PBS).

Samples to be assayed were added at appropriate dilution to these wells and incubated with 200
25 micro liter/well of 1:1000 dilution of horseradish peroxidase (HRP)-conjugated F(ab')₂ fraction of affinity-purified goat anti-human IgG or IgM antibody (Tago). The plates were then washed, and 100 micro liters/well of o-phenylenediamine (Sigma Chemical Co., St. Louis, MO) solution (0.6 mg/ml in citrate-phosphate buffer with pH 5.5 and 0.045% hydrogen peroxide). Color development was stopped with 2 N sulfuric acid. Absorbance at 490 nm was measured with an
30 automated ELISA plate reader.

Test and control samples were run in triplicate and the values of absorbance were compared to those obtained with known IgG or IgM standards run simultaneously with the supernatant samples to generate the standard curve using which the concentrations of Ig in the culture supernatant were quantitated. Data are expressed as ng/ml of Ig \pm SEM of either triplicate or quadruplicate cultures.

Immunoprecipitation Analysis and SDS PAGE. Cells were surface-labeled with ^{125}I and subjected to immunoprecipitation analysis. Briefly, PHA-activated T cells were surface-labeled with ^{125}I using lactoperoxidase and H_2O_2 as described by Vitetta et al., *J. Exp. Med.* 134:242 (1971), incorporated by reference herein. SDS-PAGE chromatography was performed on linear acrylamide gradients gels with stacking gels of 5% acrylamide. Gels were stained with Coomassie Blue, destained, and photographed or dried and exposed to X ray film (Kodak XAR-5).

Binding Assays. B7Ig was labeled with ^{125}I to a specific activity of approximately 2×10^6 cpm/pmole. Ninety-six well plastic dishes were coated for 16-24 hrs with a solution containing CTLA4Ig (0.5 micro g in a volume of 0.05 ml of 10 mM Tris, pH 8). Wells were blocked with binding buffer (DMEM containing 50 mM BES (Sigma Chemical Co.), pH 6.8, 0.1% BAS, and 10% FCS) before addition of a solution (0.09 ml) containing ^{125}I B7Ig (approximately 5×10^5 cpm) in the presence or absence of competitor. Following incubation for 2-3 hrs at 23 degrees C, wells were washed once with binding buffer, and four times with PBS. Bound radioactivity was then solubilized by addition of 0.5N NaOH, and quantified by gamma counting.

Binding to B7Ig. The functional activity of the OMCTLA4 construct encoding the complete human CTLA4 DNA gene, is shown in the experiment shown in Figure 4. COS cells were transfected with expression plasmids CD7, OMCD28 and OMCTLA4 as described above. Forty-eight hours following transfection, cells were collected and incubated with medium only (no addition) or with mAbs 9.3, B7Ig, CD5Ig or G3-7. Cells were then washed and binding was detected by a mixture of FITC-conjugated goat anti-mouse Ig and FITC-conjugated goat anti-human Ig second step reagents. Transfected cells were tested for expression of the appropriate

cell surface markers by indirect immunostaining and fluorescence was measured using FACS^R analysis as described above.

5 As shown in Figure 4, mAb 9.3 bound to CD28-transfected COS cells, but not to CTLA4-transfected cells. In contrast, the B7Ig fusion protein (but not control CD5Ig fusion protein) bound to both CD28- and CTLA4-transfected cells. CD7-transfected COS cells bound neither mAb 9.3 nor either of the fusion proteins. This indicates that CD28 and CTLA4 both bind the B cell activation antigen, B7. Furthermore, mAb 9.3 did not detectably bind CTLA4.

10 Binding of CTLA4Ig on B7 Positive CHO cells. To further characterize the binding of CTLA4Ig and B7, the binding activity of purified CTLA4Ig on B7⁺ CHO cells and on a lymphoblastoid cell line (PM LCL) was measured in the experiment shown in Figure 5. Amplified transfected CHO cell lines and PM LCLs were incubated with medium only (no addition) or an equivalent concentration of human IgC α 1-containing proteins (10 micro grams/ml) of CD5Ig, CD28Ig or CTLA4Ig. Binding was detected by FACS^R following addition of FITC-15 conjugated goat anti-human Ig second step reagents. A total of 10,000 stained cells were analyzed by FACS^R.

20 As shown in Figure 5, CD28Ig bound to B7⁺ CHO cells but not to PM LCL, a cell line which expresses relatively low levels of the B7 antigen (Linsley et al., *supra*, 1990). CTLA4Ig bound more strongly to both cell lines than did CD28Ig, suggesting that it bound with higher affinity. Neither CD28Ig nor CTLA4Ig bound to CD28⁺ CHO cells.

25 Affinity of Binding of CTLA4Ig and B7Ig. The apparent affinity of interaction between CTLA4Ig and B7Ig was then measured using a solid phase competition binding assay. Ninety-six well plastic dishes were coated with CTLA4Ig as described above. B7Ig was radiolabeled with ¹²⁵I (5 X 10⁵ cpm, 2 X 10⁶ cpm/pmole), and added to a concentration of 4 nM in the presence of the indicated concentrations (Figure 6) of unlabeled chimeric mAb L6, mAb 9.3, mAb BB-1 or B7Ig. Plate-bound radioactivity was determined and expressed as a percentage of 30 radioactivity bound to wells treated without competitor (28,300 cpm). Each point represents the mean of duplicate determinations; replicates generally varied from the mean by $\leq 20\%$.

Concentrations were calculated based on a M_r of 75,000 per binding site for mAbs and 51,000 per binding site for B7Ig.

As shown in Figure 6, only mAb BB-1 and unlabeled B7Ig competed significantly for ^{125}I -B7Ig binding (half maximal effects at approximately 22 nM and approximately 175 nM, respectively). Neither chimeric mAb L6, nor mAb 9.3 competed effectively at the concentrations tested. In other experiments, the concentrations of mAb 9.3 used were sufficient to inhibit binding of ^{125}I -B7Ig to immobilized CD28Ig or to cell surface expressed CD28 by $\geq 90\%$.

When the competition data from Figure 6 were plotted in a Scatchard representation, a dissociation constant, K_d , of approximately 12 nM was calculated for binding of ^{125}I -B7 to immobilized CTLA4Ig (Figure 7). This value is approximately 20 fold lower than the previously determined K_d of binding between ^{125}I -B7Ig and CD28 (approximately 200 nM) (Linsley et al, (1991), supra) indicating that CTLA4 is a higher affinity receptor for the B7 antigen than CD28 receptor.

To identify the molecule(s) on lymphoblastoid cells which bound CTLA4Ig, ^{125}I -surface labeled cells were subjected to immunoprecipitation analysis (Figure 8). B7⁺ CHO and PM LCL cells were surface-labeled with ^{125}I , and extracted with a non-ionic detergent solution as described above. Aliquots of extracts containing approximately 1.5×10^7 cpm in a volume of 0.1 ml were subjected to immunoprecipitation analysis as described above with no addition, or 2 micro grams each of CD28Ig, CTLA4Ig or CD5Ig. Washed immunoprecipitates were then analyzed by SDS-PAGE (10-20% acrylamide gradient) under reducing conditions. The gel was then dried and subjected to autoradiography. The left panel of Figure 8 shows an autoradiogram obtained after a 1 day exposure. The right panel of Figure 8 shows an autoradiogram of the same gel after a 10 day exposure. The autoradiogram in the center panel of Figure 8 was also exposed for 10 days. Positions of molecular weight standard are also indicated in this figure.

As shown by Figure 8, a diffusely migrating (M_r approximately 50,000 - 75,000; center at approximately 60,000) radiolabeled protein was immunoprecipitated by CTLA4Ig, but not by CD28Ig or CD5Ig. This molecule co-migrated with B7 immunoprecipitated from B7⁺ CHO

cells by CTLA4Ig, and much more weakly, by CD28Ig. These findings indicate that CTLA4Ig binds a single protein on lymphoblastoid cells which is similar in size to the B7 antigen.

Inhibition of Immune Responses In Vitro by CTLA4Ig

Inhibition of Proliferation: Previous studies have shown that the anti-CD28 mAb, mAb 9.3, and the anti-B7 mAb, mAb BB-1, inhibit proliferation of alloantigen specific T_h cells, as well as immunoglobulin secretion by alloantigen-presenting B Cells (Damle, et al., Proc. Natl. Acad. Sci. 78:5096 (1981); Lesslauer et al., Eur. J. Immunol. 16:1289 (1986)). Because CTLA4 is a high affinity receptor for the B7 antigen as demonstrated herein, soluble CTLA4Ig was tested for its ability to inhibit these responses. The effects of CTLA4Ig on T cell proliferation were examined in the experiment shown in Figure 9.

Primary mixed lymphocyte reaction (MLR) blasts were stimulated with irradiated T51 lymphoblastoid cells (LC) in the absence or presence of concentrations of murine mAb 9.3 Fab fragments, or B7Ig, CD28Ig or CTLA4Ig immunoglobulin C α fusion proteins. Cellular proliferation was measured by [3 H]-thymidine incorporation after 4 days and is expressed as the percentage of incorporation by untreated cultures (21,000 cpm). Figure 9 shows the mean of quadruplicate determinations (SEM \leq 10%).

As shown in Figure 9, CTLA4Ig inhibited the MLR reaction in a dose-dependant fashion by a maximum of > 90% with a 1/2 maximal response at approximately 30 ng/ml (approximately 0.8 nM). The Fab fragment of mAb 9.3, which previously was shown to be a more potent inhibitor of MLR than whole mAb 9.3 (Damle et al., J. Immunol. 140:1753-1761 (1988)), also inhibited the MLR, but at higher concentrations (approximately 800 ng/ml or approximately 30 nM for 1/2 maximal response). B7Ig and CD28Ig did not significantly inhibit the MLR even at higher concentrations. In another experiment, addition of B7Ig together with CTLA4Ig partially overcame the inhibition of MLR by CTLA4Ig, indicating that the inhibition was specifically due to interactions with B7 antigen.

Inhibition of Immunoglobulin Secretion. The effects of CTLA4Ig on helper T cell (T_h)-induced immunoglobulin secretion were also examined (Figure 10). $CD4^+$ T cells were mixed with allogeneic $CD19^+$ B cells in the presence or absence of the indicated immunoglobulin molecules as described above. Murine mAbs OKT8, 9.3 and BB-1 were added at 20 micro grams/ml, and Ig fusion proteins at 10 micro grams/ml. After 6 days of culture, concentrations of human IgM (SEM < 5%) in culture supernatants were determined by enzyme immunoassay (ELISA) as described above. IgM production by B cells cultured in the absence of $CD4^+$ T cells was 11 ng/ml.

As shown in Figure 10, $CD4^+$ T cells stimulated IgM production by allogenic $CD19^+$ B Cells (in the absence of $CD4^+$ T cells, IgM levels were reduced by 93%). mAbs 9.3 and BB-1 significantly inhibited T_h -induced IgM production (63% and 65% inhibition, respectively). CTLA4Ig was even more effective as an inhibitor (89% inhibition) than were these mAbs. Inhibition by control Ig molecules, mAb OKT8 and CD5Ig, was much less ($\leq 30\%$ inhibition). None of these molecules significantly inhibited Ig production measured in the presence of Staphylococcal aureus enterotoxin B. Similar results were obtained with $CD4^+$ T cells and B cells derived from other donors. These results indicate that the inhibition by CTLA4Ig is specific.

The above data also demonstrate that the CTLA4 and CD28 receptors are functionally as well as structurally related. Like CD28, CTLA4 is also a receptor for the B cell activation antigen, B7. CTLA4Ig bound ^{125}I -B7 with an affinity constant, K_d , of approximately 12 nM, a value some 20 fold higher than the affinity between CD28 and B7Ig (approximately 200 nM). Thus, CTLA4 and CD28 may be thought of as high and low affinity receptors, respectively, for the same ligand, the B7 antigen.

The apparent affinity between CD28 and B7 is similar to the affinity reported for binding of soluble alloantigen to the T cell receptor of a murine T cell hybridoma (approximately 100 nM; Schnek et al., Cell 56:47 (1989)), and is higher affinity than interactions between CD2 and LFA3 (Recny et al., J. Biol. Chem. 265:8542 (1990)), or CD4 and MHC class II molecules (Clayton et al., Nature 339:548 (1989)). The affinity constant, K_d , between CTLA4 and B7 is even greater,

and compares favorably with higher affinity mAbs (K_d 2-10,000 nM; Alzari et al., Ann. Rev. Immuno. 6:555 (1988)). The K_d between CTLA4 and B7 is similar to or greater than K_d values of integrin receptors and their ligands (10-2000 nM; Hautanen et al., J. Biol. Chem. 264:1437-1442 (1989); Di Minno et al., Blood 61:140-148 (1983); Thiagarajan and Kelley, J. Biol. Chem. 263:3035-3038 (1988)). The affinity of interaction between CTLA4 and B7 is thus among the highest yet reported for lymphoid adhesion systems.

These results demonstrate the first expression of a functional protein product of CTLA4 transcripts. CTLA4Ig, a fusion construct containing the extracellular domain of CTLA4 fused to an IgC α 1 domain, forms a disulfide-linked dimer of M_r approximately 50,000 subunits. Because no interchain disulfides would be predicted to form in the Ig portion of this fusion, it seems likely that cysteines from CTLA4 are involved in disulfide bond formation. The analogous CD28Ig fusion protein (Linsley et al, supra, 1991) also contains interchain disulfide linkage(s). These results suggest that CTLA4 receptor, like CD28 (Hansen et al., Immunogenetics 10:247-260 (1980)), exists on the T cell surface as a disulfide linked homodimer. Although CD28 and CTLA4 are highly homologous proteins, they are immunologically distinct, because the anti-CD28 mAb, mAb 9.3, does not recognize CTLA4 (Figures 4 and 5).

It is not known whether CTLA4 can activate T cells by a signalling pathway analogous to CD28. The cytoplasmic domains of murine and human CTLA4 are identical (Dariavach et al., supra 1988), suggesting that this region has important functional properties. The cytoplasmic domains of CD28 and CTLA4 also share homology, although it is unclear if this is sufficient to impart similar signaling properties to the two molecules.

CTLA4Ig is a potent inhibitor of in vitro lymphocyte functions requiring T cell and B cell collaboration (Figures 9 and 10). These findings, together with previous studies, indicate the fundamental importance of interactions between B7 antigen and its counter-receptors, CD28 and/or CTLA4, in regulating both T and B lymphocyte responses. CTLA4Ig should be a useful reagent for future investigations on the role of these interactions during immune responses. CTLA4Ig is a more potent inhibitor of in vitro lymphocyte responses than either mAb BB-1 or mAb 9.3 (Figures 9 and 10). The greater potency of CTLA4Ig over mAb BB-1 is most likely

due to the difference in affinities for B7 between these molecules (Figure 6). CTLA4Ig is also more potent than mAb 9.3, probably because, unlike the mAb, it does not also have direct stimulatory effects on T cell proliferation (June et al., Immunology Today 11:211 (1989)) to counteract its inhibitory effects. The immunosuppressive effects of CTLA4Ig in vitro suggest that future investigations are warranted into possible therapeutic effects of this molecule for treatment of autoimmune disorders involving aberrant T cell activation or Ig production.

As will be apparent to those skilled in the art to which the invention pertains, the present invention may be embodied in forms other than those specifically disclosed above without departing from the spirit or essential characteristics of the invention. The particular embodiments of the invention described above, are, therefore, to be considered as illustrative and not restrictive. The scope of the present invention is as set forth in the appended claims rather than being limited to the examples contained in the foregoing description.

EXAMPLE 5

The following provides a description of the methods used to inhibit graft versus host disease in mice receiving human islet grafts.

Female BALB/c (H-2^d) and C57BL/6 (H-2^b) mice, 6 to 8 wk. of age were obtained from The Jackson Laboratory (Bar Harbor, ME).

Human pancreatic islets cells were purified after collagenase digestion as described (C. Ricordi et al. Transplantation 52:519 (1991); A. G. Tzakis et al. Lancet 336:402 (1990); C. Ricordi, P. E. Lacy, E. H. Finke, B. J. Olack, D. W. Scharp, Diabetes 37:413 (1988)).

B6 or B10 mice, treated with streptozocin (175 mg per kilogram of body weight) 3 to 5 days before transplant and exhibiting nonfasting plasma glucose levels of greater than 280 mg/dl (with the majority over 300 mg/ml), were used as recipients.

Each animal received approximately 800 fresh human islets of 150 micro meters in diameter beneath the left renal capsule (D. Faustman and C. Coe, Science 252:1700 (1991); Y. J. Zeng et al. Transplantation 53:277 (1992)). Treatment was started immediately after transplantation.

- 5 Control animals were treated with PBS (solid lines) or L6 (dotted lines) at 50 micro grams every other day for 14 days immediately after transplantation (Figure 11A). L6 is a human IgG1 chimeric Mab. Islet transplants were considered rejected when glucose levels were greater than 250 mg/dl for three consecutive days. Animals treated with PBS (n = 14) and L6 (n = 8) had mean graft survivals of 5.6 and 6.4 days, respectively.

- 10 Animals were treated with 10 micro grams of CTLA4Ig for 14 consecutive days immediately after transplant (n = 7) (Figure 11B). Three out of seven animals maintained their grafts for >80 days. The remaining four animals had a mean graft survival of 12.75 days.

- 15 Animals were treated with 50 micro grams of CTLA4Ig every other day for 14 days immediately after human islet transplantation (Figure 11C). All animals (n = 12) treated with this dose maintained grafts throughout the analysis (Figure 11C). Selected mice were nephrectomized on days 21 and 29 after the transplant to assess the graft's function (Figure 11C).

- 20 Histology was performed on kidneys transplanted with human islet cells (Figures 12 A, B, C, D). The slides were analyzed blindly.

- Hematoxylin and eosin staining of a control human islet grafted mouse 29 days after transplantation showed a massive lymphocyte infiltration (Figure 12A). The same tissue, stained
25 for insulin, showed no detectable insulin production (Figure 12B).

- Histological examination of tissue from a CTLA4Ig-treated mouse 21 days after transplant showed intact islets under the kidney capsule with very few lymphocytes infiltrating the transplanted tissue (Figure 12C). The tissue was stained with hematoxylin and eosin. The same
30 tissue from the CTLA4Ig-treated mouse, stained for insulin, showed the production of insulin by the grafted islets (Figure 12D). Similar results were observed in graft tissue examined at later

time points. The upper, middle, and lower arrowheads identify the kidney capsule, islet transplant, and kidney parenchyma, respectively.

5 In the histopathology assay all tissues were fixed in 10% buffered formalin and processed, and 5-micro meter sections were stained either with hematoxylin and eosin or for insulin with the avidin-biotin-peroxidase method (S. M. Hsu, L. Raine, H. Fanger, J. Histochem, Cytochem, 29:577 (1981)). Magnification was x 122.

10 In Figure 13 streptozotocin-treated animals were transplanted as described hereinabove for Figure 11. The mice were treated either with PBS (dotted lines) or with MAb to human B7 (solid lines) at a dose of 50 micro grams every other day for 14 days (Figure 13). Control animals (treated with PBS) (n = 3) had a mean graft survival of 3.5 days, whereas anti-B7-treated animals (n = 5) maintained grafts from 9 to >50 days (Figure 13).

15 In Figure 14 normal glycemic, CTLA4Ig-treated, transplanted mice (dotted lines) were nephrectomized on day 44 after transplant and immediately retransplanted with either 1000 first party donor islets (dotted lines, solid circles) or 1000 second party islets (dotted lines, open circles) beneath the remaining kidney capsule.

20 These islets, frozen at the time of the first transplant, were thawed and cultured for 3 days before transplant to ensure islet function. B10 mice that had been treated with streptozotocin and exhibited nonfasting glucose levels of greater than 280 mg/dl were used as controls (solid lines) (Figure 14). No treatment was given after transplantation.

25 Control animals rejected both the first party (solid lines, closed circles) and the second party (solid lines, open circles) islet grafts by day 4 after transplant (Figure 14). The CTLA4Ig-treated mice retransplanted with second party islets had a mean graft survival of 4.5 days, whereas animals retransplanted with first party donor islets maintained grafts for as long as analyzed (>80 days) (Figure 14).

30

CTLA4Ig significantly prolongs human islet graft survival in mice in a donor-specific manner
thereby providing an approach to immunosuppression

5 C57BL/6 (B6) or C57BL/10 (B10) mice were treated with streptozotocin to eliminate mouse pancreatic islet B cell function. Diabetic animals were grafted under the kidney capsule, and treatment was started immediately after surgery. Survival of the islet grafts was monitored by the analysis of blood glucose concentrations.

10 Transplanted control animals, treated with either phosphate-buffered saline (PBS)(n = 14) or L6 (a human IgG1 chimeric MAb; n = 8), had a mean graft survival of 5.6 and 6.4 days, respectively (Figure 11A).

15 In contrast, islet rejection was delayed in animals treated with CTLA4Ig (10 micro grams per day for 14 days), with four out of the seven animals exhibiting moderately prolonged mean graft survival (12.75 days), whereas the remaining three animals maintained normal glucose levels for >80 days (Figure 11B). This eventual increase in glucose concentration may be a result of islet exhaustion because no evidence of active cellular rejection was observed.

20 In the three mice that maintained long-term islet grafts, the transient increase in glucose concentrations around day 21 after the transplant may have represented a self-limited rejection episode consistent with the pharmacokinetics of CTLA4Ig clearance after therapy (P. S. Linsley et al., Science 257:792 (1992)).

25 In subsequent experiments, the dose of CTLA4Ig was increased to 50 micro grams per animal every other day for about 14 days. This treatment resulted in 100% of the animals maintaining normal islet function throughout the experiment with no signs of a rejection crisis (Figure 11C).

30 In order to confirm that insulin production originated from the transplanted islets and not from the native mouse pancreas, we nephrectomized selected animals at days 21 and 29 to remove the islet grafts (Figure 11C). In these animals, glucose concentrations increased to above 350 mg/dl within 24 hours, which indicated that the islet xenograft was responsible for maintaining normal

glucose levels. It appears that the blocking of the CD28-B7 interaction inhibits xenogenic islet graft rejection.

The effects of treatment with the soluble receptor, namely CTLA4Ig fusion protein, were not a result of Fc binding (L6 did not effect graft rejection) or general effects on T cell or B cell function in vivo.

Histological analyses of islet xenograft from control (PBS treated) and CTLA4Ig treated mice were done (Figures 12A, 12B, 12C, 12D). The islet tissue from the control animal demonstrated evidence of immune rejection, with a marked lymphocytic infiltrate into the graft and few remaining islets (Figure 12A).

Immunohistochemical staining showed that insulin-positive cells were present only rarely, and no somatostatin-positive cells were present at all (Figure 12B). In contrast, transplant tissue from the CTLA4Ig-treated mice was devoid of any lymphocytic infiltrate (Figure 12C).

The grafts were intact, with many islets visible. In addition, the B cells observed in the human islet tissue produced human insulin (Figure 12D) and somatostatin.

The human CTLA4Ig used in this study reacts with both murine and human B7. One advantage of the xenogeneic transplant model is the availability of a MAb to human B7 that does not react with mouse B7 (T. Yokochi, R. D. Holly, E. A. Clark, J. Immunol. 128:823 (1982)). Thus, the role of human B7-bearing antigen-presenting cells (APCs) could be directly examined.

The mice were transplanted as described and then treated with 50 micro grams of MAb to human B7 every other day for 14 days after transplant. This treatment prolonged graft survival in treated mice (9 to >50 days) in comparison to that for control mice (Figure 13). The anti-B7 MAb is unable to block rejection as effectively as CTLA4Ig.

The CTLA4Ig therapy resulted in graft acceptance in the majority of mice. However, the animals may not be tolerant. Transient immunosuppression can lead to permanent islet graft

acceptance because of graft adaptation (the loss of immunogenicity as a result of the loss of APC function) (L. Hao, Y. Wang, R. G. Gill, K. J. Lafferty, *J. Immunol.* 139:4022 (1987); K. J. Lafferty, S. J. Prowse, M. Simeonovic, *Annu. Rev. Immunol.* 1:143 (1983)).

5 In order to differentiate between these possibilities, we nephrectomized selected xenografted, CTLA4Ig-treated mice (day 40) and retransplanted them under the remaining kidney capsule with either the original donor islets (first party) or unrelated second party human islets (Figure 14).

10 Streptozotocin-treated control animals, having never received an islet graft, were also transplanted with either first or second party islets. No treatment after the transplant was given. Control animals rejected the first and second party islets by day 4. The CTLA4Ig-treated animals that had received the second party islets rejected these islets by day 5, whereas animals receiving first party donor islets maintained the grafts for >80 days (Figure 14).

15 These results suggest that the CTLA4Ig treatment resulted in prolonged donor-specific unresponsiveness to the xenogeneic islets. The ability of the murine immune response to distinguish differences among the human islet donors also supports the direct recognition of the polymorphic MHC products expressed on the human islet cells.

20

EXAMPLE 6

25 The following provides a description of the methods used to induce a highly specific immune response in an animal subject.

Female BALB/c (H-2^d) and C57BL/6 (H-2^d) mice, 6 to 8 wk. of age were obtained from The Jackson Laboratory (Bar Harbor, ME).

Monoclonal antibody 11B11 is a rat IgG1 anti-murine IL-4 (Ohara, J., and W. E. Paul, 1985, Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor-1. *Nature* 315:333) (Verax (Lebanon, NH)).

5 BALB/c mice (five per group) were immunized intravenously with 10^8 SRBC alone or together with 200 micro grams chimeric L6 mAb or human CTLA4Ig fusion protein. The indicated groups were treated 2 hrs. prior to injection of SRBCs by intraperitoneal injection of 2 mls of either rat immunoglobulin or rat anti-murine IL-4 mAb 11B11 at 5 mg/ml. Treatment with chimeric L6 mAb or CTLA4Ig was repeated daily for 4 additional days.

10 All animals were given intravenous injections of SRBCs (Figure 15) or KLH (Figure 16) on day 46. Specifically, in Figure 15, the closed circle represents mice that were administered with only SRBC at day 0 and day 46. The open circle represents mice administered with only SRBC at day 46. The remaining mice represented in Figure 15 were further administered with SRBC at
15 day 46. In contrast, in Figure 16, the mice were administered with a different immunogen, KLH, at day 46 only.

Serum concentrations of mice measured as having antibodies directed against SRBCs or KLH were determined by ELISA as described (Linsley et al., *Science* 1992).

20 Serum antibody titers were calculated as the dilution giving an A_{450} of five times background. Serum antibody titer values from Figure 15 were determined from pooled sera from five mice per group, while serum antibody titer values from Figure 16 represents mean titers of five individual sera. Arrows indicate an SRBC or KLH injection at day 46.

25 Figures 15 and 16 show that the immunological response in mice injected concurrently with both CTLA4Ig and anti-IL4 (open triangle) is suppressed in an antigen-specific manner.

30 Figure 15 shows that there is no rise in serum antibody titer (i.e. no primary or secondary immunological response) in mice injected concurrently with CTLA4Ig and anti-IL4 and injected with SRBC at day 0 and day 46. The combination of CTLA4Ig and anti-IL4 suppresses a

primary and secondary immune response and induces long lasting immunological non-responsiveness to SRBC.

5 Additionally, Figure 15 shows that there is no primary immunological response in mice injected concurrently with CTLA4Ig and the control rat Ig (Cappel, Organon-Technika, Palo Alto, CA). However, these mice exhibit a secondary immunological response after injection with SRBC at day 46 (closed triangle, Figure 15).

10 Figure 16 shows that administration of CTLA4Ig and anti-IL4, followed by a different immunogen, KLH, at day 46 in mice does not suppress a primary immune response to KLH in mice. Instead, these mice exhibited a primary immune response to KLH (open triangle, Figure 16). Thus, mice treated with CTLA4Ig and anti-IL4 exhibited a highly specific immune response depending on the antigen administered therein.

15 EXAMPLE 7

The following provides a description of the methods used to determine the regions within CTLA4 which are required for binding B7-1 (e.g., CD80), using CTLA4/CD28 chimeric molecules.

20 The regions in CTLA4Ig which are required for its high avidity binding to B7-1 have been identified by site-specific and homolog mutagenesis. The following is a description of how to make soluble CTLA4/CD28 hybrid fusion proteins which bind B7.

25 MATERIALS AND METHODS

Monoclonal antibodies (mAbs). Murine mAb's specific for CTLA4 were prepared and characterized as previously described (Linsley et al. J. Ex. Med., (1992) 176:1595-1604). Antibody 9.3 (anti-CD28) has been described previously ((Hansen et al., Immunogenetics
30 10:247-260 (1980)).

Cell Culture. The preparation of stably transfected B7-1 positive CHO cells has been previously described (Linsley et al., in J. Exp. Med. 173:721-730 (1991); P. S. Linsley et al., J. Exp. Med. 174:561 (1991)).

- 5 Cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS), 0.2mM proline, and 1micro M methotrexate. COS cells were grown in DMEM supplemented with 10% FBS. CTLA4Ig was prepared in CHO cells as previously described (Example 1).

10 *CTLA4Ig and CD28Ig site-directed mutant expression plasmids.* Site-directed mutagenesis was performed on a vector encoding soluble chimeric form of CTLA4 (CTLA4Ig) in which the extracellular domain of CTLA4 was genetically fused to the hinge and constant regions of a human IgG heavy chain (Example 1). CTLA4Ig site-directed mutants were prepared by encoding the desired mutation in overlapping oligonucleotide primers and generating the mutants by PCR (Ho et al., 1989, supra.) using the CTLA4Ig plasmid construct as a template.

- 15 Six mutants were prepared which encoded substitutions to alanine in the highly conserved hexapeptide 97MYPPPY102 forming part of the putative CDR3-like domain (Figures 17 and 22) (Ho et al., 1989, supra.).

- 20 In addition, two mutants encoding the residues P103A and Y104A (MYPPAY and MYPPPA, respectively) from the CD28Ig 99MYPPPY104 hexapeptide using CD28Ig as a template were also prepared by the same method.

25 Primers required for PCR reactions but not for introducing mutations included (1) a CDM8 forward (CDM8FP) primer encoding a complementary sequence upstream of the HindIII restriction site at the 5' end of the CDM8 stuffer region, and (2) a reverse primer (CDM8RP) encoding a complementary sequence downstream of the XbaI site at the 3' end of the CDM8 stuffer region.

- 30 These primers encoded the following sequences:

CDM8FP:5'-AATACGACTCACTATAGG

CDM8RP:5'-CACCACACTGTATTAACC

PCR conditions consisted of 6 min at 94 degrees C followed by 25 cycles of 1 min at 94 degrees C, 2 min at 55 degrees C and 3 min at 72 degrees C. Taq polymerase and reaction conditions were used as suggested by the vendor (Perkin Elmer Cetus, Emeryville, CA). PCR products were digested with HindIII and XbaI and ligated to HindIII/XbaI-cut CDM8 expression vector.

To confirm that the desired mutations had been inserted and to verify the absence of secondary mutations, each CTLA4Ig mutant fusion protein (an example of a soluble CTLA4 mutant fusion protein) was sequenced by the dideoxy chain termination/extension reaction with Sequenase reagents used according to the manufacturers recommendations (United States Biochemical Corp., Cleveland, OH).

Plasmids were transfected into COS cells (Aruffo et al., Cell 61:1303 (1990)) and the conditioned media was used as a source for the resulting Ig mutant fusion proteins.

CTLA4/CD28Ig hybrid expression plasmids. CTLA4/CD28Ig hybrid plasmids encoding the constructs HS2, HS4, HS4-A, HS4-B, and HS5 (Figure 19) were prepared by PCR using overlapping oligonucleotide primers designed to introduce CTLA4 sequences into CD28Ig while, at the same time, deleting the equivalent region from CD28. The same CDM8 forward and reverse PCR primers described above were also used.

Each cDNA construct was genetically linked to cDNA encoding the hinge and constant regions of a human IgG1 in order to make soluble chimeras.

A HS6 hybrid was prepared in a similar manner to that described above except that the CDR1-like region in CTLA4Ig was replaced with the equivalent region from CD28Ig.

HS7, HS8, and HS9 constructs were prepared by replacing a \square 350 base-pair HindIII/HpaI 5' fragment of HS4, HS4-A, and HS4-B, respectively, with the equivalent cDNA fragment

similarly digested from HS5 thus introducing the CDR1-like loop of CTLA4 into those hybrids already containing the CTLA4 CDR3-like region.

5 HS10-HS13 constructs are domain homolog mutants which were prepared by introducing the CDR2-like loop of CTLA4Ig into previously constructed homolog mutants. This was done by overlapping PCR mutagenesis whereby primers were designed to introduce CTLA4 CDR2-like sequences into homolog templates while at the same time deleting the equivalent CD28 CDR2-like region from the molecule.

10 Accordingly, HS4 served as a template to make HS10; HS7 served as a template to make HS11; HS4-A served as a template to make HS12; and HS8 served as a template to make HS13 (Figure 19). The CDM8 primers described above were also used in these constructions.

15 The HS14 hybrid construct was prepared by replacing the CDR2-like loop of CD28 with the equivalent loop from CTLA4Ig (Figure 19).

Oligonucleotide primers designed to introduce these changes were used in overlapping PCR mutagenesis identical to that described for other mutants.

20 PCR reactions and subcloning into CDM8 were performed as described above. Again all mutants were sequenced by the dideoxy chain termination/extension reaction.

25 Plasmids encoding each of the mutants were transfected into COS cells and the resulting soluble Ig fusion proteins were quantitated in culture media and visualized by Western blot as described in following sections.

Quantitation of the resulting Ig fusion proteins in culture media. Soluble mutant fusion proteins were quantitated in an enzyme immunoassay by determining the amount of Ig present in serum-free COS cell culture media.

Microtiter plates (Immulon2; Dynatech Labs., Chantilly, VA) were coated with 0.5 micro g/ml goat anti-human IgG (Jackson Immunoresearch Labs., West Chester, PA) for 16-24h at 4 degrees C. Wells were blocked for 1h with specimen diluent (Genetic Systems, Seattle, WA), then washed with PBS containing 0.05% Tween 20 (PBS-Tw).

5 COS cell culture media containing fusion proteins was added at various dilutions and incubated for 1h at 22 degrees C. Known concentrations of CTLA4Ig were also added to separate wells on each plate for a standard curve.

10 After washing, horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Tago, Burlingame, CA) diluted 1:12,000 was added and incubated for 1h at 22 degrees C. Wells were then washed and incubated with 3,3',5,5' tetramethylbenzidine (TMB) substrate (Genetic Systems) for 15 min before stopping the reaction by the addition of 1N H₂SO₄. Optical density was measured at dual wavelengths of 450 and 630 nm on a microtiter plate reader (Genetic
15 Systems).

Concentration of mutant Ig fusion protein was determined by comparison with a standard curve of known concentrations of CTLA4Ig.

20 *Immunoprecipitation and Western blot analysis.* CTLA4/CD28Ig hybrid fusion proteins present in culture media were adsorbed to protein A-Sepharose by overnight incubation at 4 degrees C. The beads were washed with PBS containing 0.1% Nonidet-P40 (NP40) then SDS PAGE sample buffer was added and the eluted protein was loaded onto an SDS polyacrylamide gel.

25 Western blot transfer of protein onto nitrocellulose was done by standard procedures. Nitrocellulose membranes were then blocked with PBS containing 0.1% NP40 and 1% non-fat dry milk powder.

30 After washing in PBS-Tw membranes were incubated with alkaline phosphatase-conjugated goat anti-human IgG (Boehringer Mannheim, Indianapolis, IN) diluted 1:1,000 and incubated for 1h at 22 degrees C. Blots were then washed and developed using standard procedures.

B7 positive CHO cell enzyme immunoassay. The ability of CTLA4Ig mutant fusion proteins, and CTLA4/CD28Ig hybrid fusion proteins to bind B7-1 stably expressed on CHO cells was determined by an enzyme immunoassay.

5

Round bottom tissue culture treated 96 well microtiter plates (Corning, Corning, NY) were seeded with B7-1 positive CHO cells at 10^3 cells/well. Two days later the confluent cells were fixed in 95% ethanol for 15 min.

- 10 After washing with PBS-Tw, mutant Ig fusion proteins were added at various concentrations and incubated for 1h at 4 degrees C. After washing, HRP-conjugated goat anti-human IgG (Tago) diluted 1:10,000 was added and incubated for 1h at 22 degrees C.

- 15 Wells were then washed and TMB substrate added as above and allowed to react for 30 min before stopping the reaction with 1N H_2SO_4 . Absorbance of the wells was measured at 450 nm.

CD28Ig site-directed mutant fusion protein binding assay. Site-directed mutant fusion proteins of CD28Ig were assayed for their ability to bind to B7-1 by an indirect enzyme immunoassay.

- 20 Wells of ELISA plates were coated with a chimeric fusion protein containing the extracellular domain of human B7-1 fused to a mouse IgG1 Fc region, at 5 micro grams/ml for 16h at 4 degrees C. Wells were blocked for 1h with specimen diluent (Genetic Systems) then washed with PBS-Tw. COS cell culture media containing known concentrations of mutant fusion protein was added at various concentrations and incubated for 1h at 22 degrees C.

25

Known concentrations of CD28Ig were also added to separate wells on each plate. After washing, HRP-conjugated goat anti-human IgG (Tago) diluted 1:10,000 was added and incubated for 1h at 22 degrees C. TMB substrate was added and optical densities read as described for quantitation of Ig fusion proteins in culture media.

30

mAb binding to Ig fusion proteins. The ability of anti-CTLA4 mAb's and the anti-CD28 mAb 9.3 to bind CTLA4/CD28Ig hybrid fusion proteins and CTLA4Ig mutant fusion proteins was assessed by an enzyme immunoassay.

- 5 Wells of microtiter plates (Immulon 2) were coated with 0.5 micro grams/ml of goat anti-human IgG (Jackson) for 16-24h at 4 degrees C. Plates were blocked for 1h with specimen diluent (Genetic Systems), washed with PBS-Tw, then incubated with the Ig fusion proteins for 1h at 22 degrees C. After washing, wells were incubated with mAb at 1 micro grams/ml for 1h at 22 degrees C.

10

After further washing, HRP-conjugated goat anti-mouse Ig (Tago) diluted 1:10,000 was added and incubated for 1h at 22 degrees C. TMB substrate was added and optical density measured as described above.

- 15 *CTLA4 molecular model.* An approximate three-dimensional model of the CTLA4 extracellular domain was generated based on the conservation of consensus residues of IGSF variable-like domains.

- 20 Using such IGSF consensus residues as "anchor points" for sequence alignments, CTLA4 residues were assigned to the A, B, C, C', C'', D, E, F, G strands of an Ig variable fold (Williams/Barclay, 1988, supra.) and the connecting loop regions (Figure 21).

- 25 The CTLA4 model was built (InsightII, Discover, Molecular Modeling and Mechanics Programs, respectively, Biosym Technologies, Inc., San Diego) using the variable heavy chain of HyHEL-5 (Sheriff et al., 1987 PNAS 84:8075-8079) as template structure. Side-chain replacements and loop conformations were approximated using conformational searching (Brucoleri et al., 1988 335:564-568).

- 30 Several versions of the model with modified assignments of some residues to α -strands or loops were tested using 3D-profile analysis (Luthy et al., 1992, Nature 336:83-85) in order to improve the initial alignment of the CTLA4 extracellular region sequence with an IGSF variable fold.

RESULTS

Construction and binding activity of CTLA4Ig and CD28Ig mutant fusion proteins. A sequence alignment of various homologues of CD28 and CTLA4 is demonstrated in Figure 17. In Figure 17, sequences of human (H), mouse (M), rat (R), and chicken (Ch) CD28 are aligned with human and mouse CTLA4. Residues are numbered from the mature protein N-terminus with the signal peptides and transmembrane domains underlined and the CDR-analogous regions noted. Dark shaded areas highlight complete conservation of residues while light shaded areas highlight conservative amino acid substitutions in all family members.

Regions of sequence conservation are scattered throughout the extracellular domains of these proteins with the most rigorous conservation seen in the hexapeptide MYPPPY motif located in the CDR3-like loop of both CTLA4 and CD28 (Figure 17). This suggests a probable role for this region in the interaction with a B7 antigen, e.g., B7-1 and B7-2.

To test this possibility, site-directed alanine scanning mutations were introduced into this region of CTLA4Ig using PCR oligonucleotide primer-directed mutagenesis thereby resulting in CTLA4Ig mutant fusion proteins. Similarly two alanine mutations were introduced into the CD28Ig MYPPPY motif thereby resulting in CD28Ig mutant fusion proteins.

All cDNA constructs were sequenced to confirm the desired mutations before transfection into COS cells. The concentrations of mutant Ig fusion proteins in serum-free COS cell culture media were determined by an Ig quantitation assay.

The ability of each CTLA4Ig mutant fusion protein to bind to B7-1 expressed on stably transfected CHO cells was then determined by an indirect cell binding immunoassay. Binding of CD28Ig mutant fusion proteins to B7-1 was assessed by an indirect enzyme immunoassay. Each of these assays are described in Materials and Methods.

Mutagenesis of each residue of the CTLA4Ig MYPPPY motif to alanine had a profound effect on binding to B7-1 as shown in Figure 18. Figure 18 shows that mutations in the MYPPPY motif of CTLA4Ig and CD28Ig disrupt binding to B7-1. Site-directed mutant Ig fusion proteins were produced in transiently transfected COS cells, quantitated and tested for their ability to bind to B7-1.

In Figure 18 fusion protein quantitations were repeated at least twice with replicate determinations. Specifically, Figure 18 shows that CTLA4Ig mutants bind to stably transfected, ethanol-fixed B7-1+ CHO cells grown to confluency in ELISA tissue culture plates. Binding data is expressed as the average of duplicate wells and is representative of at least two experiments.

Y98A and P100A mutants bound to B7-1 but with considerably reduced ability relative to wild-type CTLA4Ig. In contrast, the mutants M97A, P99A, P101A and Y102A showed an almost complete loss of binding. Furthermore, the CD28Ig MYPPPY mutants P103A and Y104A did not display detectable binding to B7-1 immobilized on wells of ELISA plates.

B7-1 transfected CHO cells which were incubated with CTLA4Ig mutant fusion protein, labeled with anti-human FITC, and assayed using a FACSCAN showed equivalent results. These results clearly demonstrate a critical role for the MYPPPY motif in both CTLA4Ig and CD28Ig binding to B7-1.

Characterization of CTLA4/CD28Ig hybrid fusion proteins. Since the MYPPPY motif is common to both CTLA4Ig and CD28Ig, it alone cannot account for the observed differences in binding to B7-1 seen with CTLA4Ig and CD28Ig. The contribution of less well conserved residues to high avidity binding B7-1 was assessed using a series of homolog mutants.

The three CDR-like regions of CD28 were replaced in various combinations with the equivalent regions from the CTLA4 extracellular domain (Figure 19). Figure 19 is a map of CTLA4/CD28Ig mutant fusion proteins showing % binding activity to B7-1+ CHO cells relative to CTLA4-Ig. Conserved cysteine residues (C) are shown at positions 21, 92 and 120

respectively (CTLA4 numbering). Also shown is the position of the MYPPPY motif. Open areas represent CD28 sequence; filled areas represent CTLA4 sequence; cross-hatched areas represent beginning of IgG Fc.

- 5 Percent binding activities were determined by comparing binding curves (Figure 20 A and B) relative to CTLA4-Ig and finding the concentration of a mutant required to give the same O.D. as that found for CTLA4-Ig. The ratio of mutant protein to CTLA4-Ig concentration at a particular O.D. was then expressed as % binding activity. At least two A450 readings were taken from the linear part of the CTLA4-Ig binding curve and the average % binding activity determined.

10

A total of 14 hybrid cDNA constructs were prepared, sequenced, and transfected into COS cells. Concentrations of Ig fusion proteins in serum-free culture media were determined and their electrophoretic mobility compared by SDS-PAGE including Western blotting analysis.

- 15 Under reducing conditions each chimeric protein migrated with a relative molecular mass ranging between that of CTLA4Ig (Mr-50kDa) and CD28Ig (Mr-70kDa) depending on the size of the exchanged region.

- 20 Under non-reducing conditions the proteins migrated primarily between 100-140kDa indicating that these fusion proteins existed as disulfide-linked dimers despite mutagenesis of the cysteine residues in the hinge region of the Fc.

- 25 Since four of the five conserved cysteine residues in CTLA4 and CD28 are thought to be involved in intrachain disulfide bonds, dimerization of the fusion proteins was therefore most likely attributable to the fifth conserved cysteine residue at position 120 in CTLA4 (position 123 in CD28).

- 30 *Binding of CTLA4/CD28Ig hybrid fusion proteins to B7-1.* The hybrid fusion proteins were tested for their ability to bind to B7-1 by the same indirect cell binding immunoassay used to assay the site-specific CTLA4Ig and CD28Ig mutant fusion proteins.

Under these conditions the binding between CD28Ig and B7-1 is barely detectable (Figures 20 A and B). However, replacing residues 97 to 125 (the CDR3-like extended region) of CD28 with the corresponding residues of CTLA4 resulted in an approximately two and a half orders of magnitude increase in binding of the CD28Ig analog to B7-1 (Figure 20 A and B). Figures 20 A and B show that CTLA4/CD28Ig mutant fusion proteins demonstrate involvement of CDR-analogous regions in high avidity binding to B7-1 CHO cells. Mutants were assayed as described in Example 4. Data is expressed as the average of duplicate wells and is representative of at least three experiments. From these curves % binding activity relative to CTLA4-Ig was determined as explained and shown in Figure 19.

Binding to B7-1 by this construct, termed HS4 (Figure 19), is approximately five fold less than wild type CTLA4Ig. The HS2 hybrid which includes additional N-terminal residues of CTLA4 (amino acids 1-21), did not improve the ability of the hybrid molecule to bind to B7-1 relative to HS4.

The HS6 construct which represents the CTLA4Ig sequence except that it contains the CDR1-like region of CD28 (residues 25-32), bound similarly. However, the additional inclusion of the CTLA4 CDR1-like region (residues 24-31) into the HS4 construct (termed HS7), showed further improved binding so that the binding affinity is approximately 44% of CTLA4Ig (Figure 19).

In contrast, inclusion of the CDR2-like region of CTLA4 (residues 50-57) into HS4 (construct HS10), did not further increase binding (Figure 19). A similar result was found for construct HS11 which had all three CDR-like region sequences of CTLA4 included into CD28Ig. The HS5 hybrid which contained only the CDR1-like domain of CTLA4 bound at very low levels.

The CTLA4/CD28Ig hybrid HS4-A encoded CTLA4Ig residues 95-112 in the C-terminally extended CDR3-like region; nine CTLA4 derived residues fewer than HS4 (Figure 19). HS4-A bound B7-1 CHO cells less well than HS4 (Figures 19 and 20 B). However, addition of the CTLA4 CDR1-like loop (HS8 hybrid), increased B7-1 binding from about 2% to nearly 60% of wild type binding.

On the other hand, addition of the CTLA4 CDR2-like loop into HS4-A (HS12) did not increase binding relative to HS4-A; neither did addition of all three CTLA4 CDR-like regions (HS13, Figure 19).

- 5 Another hybrid called HS4-B, encoded the CD28 CDR3-like region including the MYPPPY motif followed by CTLA4 residues 113-122 (Figure 19).

HS4-B and HS4-A displayed similar binding to B7-1. Unlike HS4-A, however, the inclusion of the CTLA4 CDR1-like loop into HS4-B (HS9) did not improve binding (Figure 19), suggesting
10 that residues immediately adjacent to the CTLA4Ig MYPPPY motif were important determinants in high avidity binding.

Monoclonal antibody binding to CTLA4/CD28Ig hybrid fusion proteins. The structural integrity of each hybrid fusion protein was examined by assessing their ability to bind mAb's specific for
15 CTLA4 or CD28 in an enzyme immunoassay. The CTLA4 specific mAb's 7F8, 11D4 and 10A8 block ligand binding (Linsley et al. (1992) supra.).

These antibodies bound to each of the CTLA4Ig mutant fusion proteins except 11D4 which failed to bind to P99A and P101A. Since 7F8 and 10A8 bound to these mutants, the lack of
20 binding by 11D4 can probably be attributed to mutagenesis perturbing the epitope recognized by 11D4.

Conversely, each antibody failed to bind to any of the homolog hybrid fusion proteins except 7F8 which bound to HS6, and 11D4 which bound weakly to HS8. As many of these homolog
25 hybrid fusion proteins were, to some extent, able to bind to B7-1, it is likely that lack of binding by the antibodies was due to disruption of conformational epitopes formed by spatially adjacent but non-linear sequences.

The CD28 specific mAb 9.3 (Linsley et al. (1992) supra.) failed to bind to either of the CD28
30 site-directed mutant fusion proteins but bound to the hybrid fusion proteins HS4, HS4-A, HS7

and HS8. With HS2, weaker binding was observed. No binding was seen with the HS5 and HS6 constructs.

5 *CTLA4 model.* Figure 21 shows a schematic representation of the CTLA4 model. The assignment of CTLA4 residues to CDR-like regions is shown in Figure 17. The CTLA4 model suggests the presence of an additional (non-Ig) disulfide bond between residues Cys48 and Cys66 which supports the similarity of CTLA4 and the Ig variable fold.

10 The two possible N-linked glycosylation sites in CTLA4 map to solvent exposed positions of the Ig beta-strand framework regions. 3D-profile analysis indicated that the CTLA4 sequence is overall compatible with an Ig V-fold, albeit more distantly related.

15 Residue Val114 represents the last residue of the CTLA4Ig-like domain. The conformation of the region between Val114 and the membrane-proximal Cys120 which is thought to form the CTLA4 homodimer is highly variable in the CD28 family. The picture that emerges is that CD28 family members mainly utilize residues in two of three CDR-like regions for binding to B7-1.

20 The MYPPPY motif represents a conserved scaffold for binding which appears to be augmented by its C-terminal extension and which is specifically modulated by the highly variable CDR1-like region. CDR3 and CDR1-like regions are spatially contiguous in Ig-variable folds. The CDR2 like region is spatially distant and does not, in the case of the CD28 family, significantly contribute to the binding to B7-1.

	DESIGNATION	FRAMEWORK	MODIFICATIONS
	HS1	CTLA4	1-24 OF CD28 97-125 OF CD28
5	HS2	CD28	1-21 OF CTLA4 95-122 OF CTLA4
	HS3	CTLA4	97-125 OF CD28
	HS4	CD28	95-122 OF CTLA4
	HS4A	CD28	95-112 OF CTLA4
10	HS4B	CD28	113-122 OF CTLA4
	HS5	CD28	24-31 OF CTLA4
	HS6	CTLA4	25-32 OF CD28
	HS7	CD28	95-122 OF CTLA4 24-31 OF CTLA4
15	HS8	CD28	24-31 OF CTLA4 95-112 OF CTLA4
	HS9	CD28	24-31 OF CTLA4 113-122 OF CTLA4
	HS10	CD28	95-122 OF CTLA4 50-57 OF CTLA4
20	HS11	CD28	24-31 OF CTLA4 50-57 OF CTLA4 95-122 OF CTLA4
	HS12	CD28	50-57 OF CTLA4 95-112 OF CTLA4
25	HS13	CD28	24-31 OF CTLA4 50-57 OF CTLA4 95-112 OF CTLA4
	HS14	CD28	50-57 OF CTLA4
30			

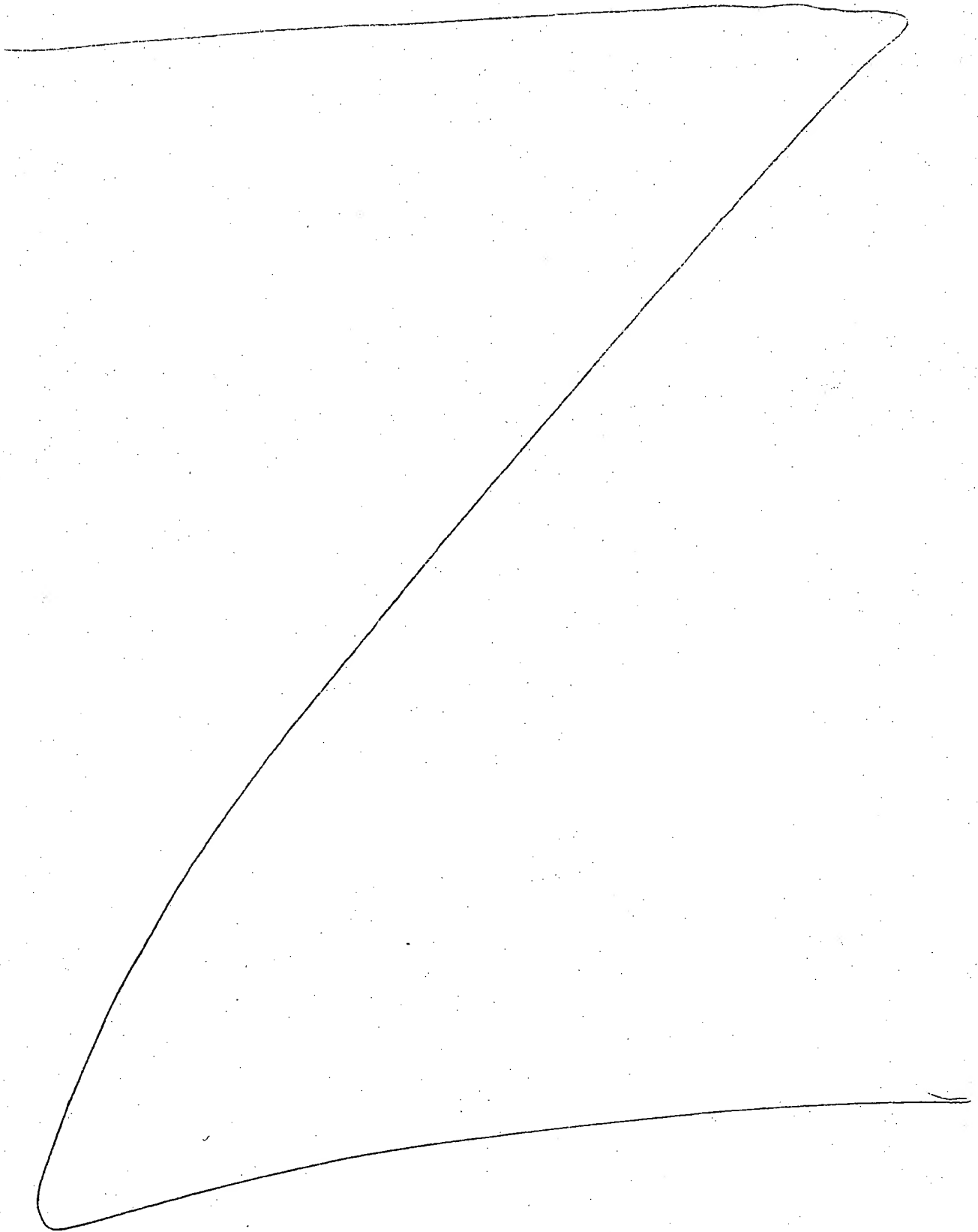




TABLE B. Binding of CTLA4 and CD28 monoclonal antibodies to CTLA4Ig and CD28Ig mutant fusion proteins and to CTLA4/CD28Ig hybrid fusion proteins.

		anti-CTLA4 mAbs		anti-CD28 mAb	
		7F8	11D4	10A8	9.3
<u>CTLA4Ig MUTANT FUSION PROTEIN</u>					
10	AYPPPY	+++	+++	+++	-
	MAPPPY	++	+	++	-
	MYAPPY	+	-	+	-
	MYPAPY	+++	+++++	+++	-
	MYPPAY	+++	-	+	-
15	MYPPPA	+++	++	+++	-
	AAPPPY	+	++	+++	-
<u>CD28Ig MUTANT FUSION PROTEIN</u>					
20	MYPPAY	-	-	-	-
	MYPPPA	-	-	-	+
<u>CTLA4/CD28Ig HYBRID FUSION PROTEINS</u>					
25	HS1	-	-	-	-
	HS2	-	-	-	+
	HS3	-	-	-	-
	HS4	-	-	-	+++
	HS5	-	-	-	-
30	HS6	+	-	-	-
	HS4-A	-	-	-	++
	HS4-B	-	-	-	++
	HS7	-	-	-	+++
	HS8	-	+	-	+++
35	HS9	-	+	-	-
	HS10	-	-	-	-
	HS11	-	-	-	+
	HS12	-	-	-	-
	HS13	-	-	-	-
40	HS14	-	-	-	-
	CTLA4Ig	+++	+++	+++	-
	CD28Ig	-	-	-	+++

Antibody binding was rated from that seen for wild type protein (+++) to above background (+), and no detectable binding (-).

EXAMPLE 8

The following provides a description of the methods used to generate the nucleotide sequences encoding the soluble CTLA4 mutant molecules having one or more substitution mutations in the extracellular CTLA4 portion. SA single-site mutants L104EIg were as generated and tested for binding kinetics for CD80 and/or CD86. Numerous single-site mutants were generated. The single-site mutants that were tested for binding to CD80 and CD86, or tested for allostimulation include the following:

10 L104EIg (Figure 23) and L104SIg. The L104EIg single-site mutant was used as a template to generate numerous double-site mutant CTLA4 sequences, including L104EA29YIg (Figure 24), L104EA29LIg (Figure 25), L104EA29Tig (Figure 26), L104EA29Wlg (Figure 27), L104EG105FIg, and L104ES25RIg. Triple-site mutants were also generated, including L104EA29YS25KIg and L104EA29YS25RIg.

15 The L104EIg nucleotide sequence was used as a template to generate the double-site mutant CTLA4 sequence, L104EA29YIg, which was tested for binding kinetics.

CTLA4Ig Codon Based Mutagenesis:

20 Single-site mutant nucleotide sequences were generated using CTLA4Ig (U. S. Patent Nos: 5,844,095; 5,851,795; and 5,885,796) as a template. Mutagenic CTLA4Ig oligonucleotide PCR primers were designed for random mutagenesis of a specific codon by allowing any base at positions 1 and 2 of the codon, but only guanine or thymine at position 3 (XXG/T). In this manner, a specific codon encoding an amino acid could be

25 randomly mutated to code for each of the 20 amino acids. PCR products encoding mutations in close proximity to E95-G107 of CTLA4Ig (Figure 22), were digested with SacI/XbaI and subcloned into similarly cut CTLA4Ig π LN expression vector. This method was used to generate numerous nucleotide sequences encoding single-site CTLA4 mutant molecules (Example 9). The functional properties of some of the CTLA4

30 single-site mutants was analyzed (see Example 9). Some of the single-site mutants were identified as CTLA4 mutants of interest and were sequenced (see Example 8).

L104EIg was identified as a CTLA4 mutant molecule of interest (e.g., having single-site mutation). To generate double-site CTLA4 mutant molecules, the nucleotide sequence encoding L104EIg was used as a template for mutagenesis in proximity to S25-R33 of CTLA4Ig (e.g., serine at position +25 through arginine at position +33), a silent NheI restriction site was first introduced 5' to this region, by PCR primer-directed mutagenesis. PCR products were digested with NheI/XbaI and subcloned into similarly cut CTLA4Ig or L104EIg or L104EIg expression vectors. The functional properties of some of the CTLA4 double-site mutants was analyzed (see Example 9). Some of the double-site mutants were identified as CTLA4 mutants of interest and were sequenced (see Example 8).

L104E29YIg was identified as a CTLA4 molecule of interest (e.g., having double-site mutations). The nucleotide sequence encoding L104E29YIg was used to generate triple-site CTLA mutant molecules. The functional properties of some of the CTLA4 triple-site mutants was analyzed. Some of the triple-site mutants were sequenced (see Example 8).

This method was used to generate the double-site CTLA4 mutant molecule L104EA29YIg (Figure 7).

20 **EXAMPLE 9**

The following provides a description of the methods used to identify the single- and double- and double-site mutant CTLA polypeptides, expressed from the constructs described in Example 8, that exhibited binding to CD80 and/or CD86 antigens.

25

Current *in vitro* and *in vivo* studies indicate that CTLA4Ig by itself is unable to completely block the priming of antigen specific activated T cells. *In vitro* studies with CTLA4Ig and either monoclonal antibody specific for CD80 or CD86 measuring inhibition of T cell proliferation indicate that anti-CD80 monoclonal antibody did not augment CTLA4Ig inhibition. However, anti-CD86 monoclonal antibody did, indicating that CTLA4Ig was not as effective at blocking CD86 interactions. These data support

30

earlier findings by Linsley et al. (*Immunity*, 1994, 1:793-801) showing inhibition of CD80-mediated cellular responses required approximately 100 fold lower CTLA4Ig concentrations than for CD86-mediated responses. Based on these findings, it was surmised that soluble CTLA4 mutant molecules having a higher avidity for CD86 than wild type CTLA4 should be better able to block the priming of antigen specific activated cells than CTLA4Ig.

To this end, the soluble CTLA4 mutant molecules described in Example 8 above were screened using a novel screening procedure to identify several mutations in the extracellular domain of CTLA4 that improve binding avidity for CD80 and CD86.

In general, COS cells were transfected with individual miniprep plasmid cDNA and three day conditioned culture media applied to BIAcore biosensor chips (Pharmacia Biotech AB, Uppsala, Sweden) coated with soluble CD80Ig or CD86Ig. The specific binding and dissociation of mutant proteins was measured by surface plasmon resonance (O'Shannessy, D. J., et al., 1997 *Anal. Biochem.* 212:457-468).

Screening Method

COS cells grown in 24 well tissue culture plates were transiently transfected with mutant CTLA4Ig and culture media collected 3 days later.

Conditioned COS cell culture media was allowed to flow over BIAcore biosensor chips derivatized with CD86Ig or CD80Ig, and mutant molecules were identified with off rates slower than that observed for wild type CTLA4Ig. The cDNAs corresponding to selected media samples were sequenced and DNA was prepared from these cDNAs to perform larger scale COS cell transient transfection, from which mutant CTLA4Ig protein was prepared following protein A purification of culture media.

BIAcore analysis conditions and equilibrium binding data analysis were performed as described in J. Greene et al. 1996 *J. Biol. Chem.* 271:26762.

BIAcore Data Analysis

5 Sensorgram baselines were normalized to zero response units (RU) prior to analysis. Samples were run over mock-derivatized flow cells to determine background RU values due to bulk refractive index differences between solutions. Equilibrium dissociation constants (K_d) were calculated from plots of R_{eq} versus C , where R_{eq} is the steady-state response minus the response on a mock-derivatized chip, and C is the molar concentration of analyte. Binding curves were analyzed using commercial nonlinear
10 curve-fitting software (Prism, GraphPAD Software).

Experimental data were first fit to a model for a single ligand binding to a single receptor (1-site model, i.e., a simple langmuir system, $A+B \rightleftharpoons AB$), and equilibrium association constants ($K_d=[A] \cdot [B]/[AB]$) were calculated from the equation $R=R_{max} \cdot C/(K_d+C)$.
15 Subsequently, data were fit to the simplest two-site model of ligand binding (i.e., to a receptor having two non-interacting independent binding sites as described by the equation $R=R_{max1} \cdot C/(K_{d1}+C)+R_{max2} \cdot C/(K_{d2}+C)$).

20 The goodness-of-fits of these two models were analyzed visually by comparison with experimental data and statistically by an F test of the sums-of-squares. The simpler one-site model was chosen as the best fit unless the two-site model fit significantly better ($p<0.1$).

Association and disassociation analyses were performed using BIA evaluation 2.1
25 Software (Pharmacia). Association rate constants k_{on} were calculated in two ways, assuming both homogenous single-site interactions and parallel two-site interactions. For single-site interactions, k_{on} values were calculated according to the equation $R_t=R_{eq}(1-\exp^{-k_s(t-t_0)})$, where R_t is a response at a given time, t ; R_{eq} is the steady-state response; t_0 is the time at the start of the injection; and $k_s=dR/dt=k_{on} \cdot Ck_{off}$, where C is a concentration
30 of analyte, calculated in terms of monomeric binding sites. For two-site interactions k_{on} values were calculated according to the equation $R_t=R_{eq1}(1-\exp^{-k_{s1}(t-t_0)})+R_{eq2}(1-\exp^{-k_{s2}(t-t_0)})$.

For each model, the values of k_{on} were determined from the calculated slope (to about 70% maximal association) of plots of k_s versus C .

Dissociation data were analyzed according to one site ($AB=A+B$) or two sites ($A_iB_j=A_i+B_j$) models, and rate constants (k_{off}) were calculated from best fit curves. The binding site model was used except when the residuals were greater than machine background (2-10RU, according to machine), in which case the two-binding site model was employed. Half-times of receptor occupancy were calculated using the relationship $t_{1/2}=0.693/k_{off}$.

Table I: Equilibrium binding constants (See also Figure 28)

	CD80Ig (Kd)	CD86Ig (Kd)
CTLA4Ig	6.51±1.08	13.9±2.27
L104EIg	4.47±0.36	6.06±0.05
L104EA29YIg	3.66±0.41	3.21±0.23

BIAcore™ Analysis: All experiments were run on BIAcore™ or BIAcore™ 2000 biosensors (Pharmacia Biotech AB, Uppsala) at 25°C. Ligands were immobilized on research grade NCM5 sensor chips (Pharmacia) using standard N-ethyl-N'-(dimethylaminopropyl) carbodiimideN-hydroxysuccinimide coupling (Johnsson, B., et al. 1991 *Anal. Biochem.* 198: 268-277; Khilko, S.N., et al. 1993 *J. Biol. Chem.* 268:5425-15434).

Table II: BIAcore analysis of Single-site CTLA4Ig mutants binding to CD86

Single-site Mutant:	% of Mutants that Bind:	% Mutant Non-binding:
S25	95	5
P26	94	6
G27	91	9
K28	57	43

A29	74	26
T30	95	5
E31	5	95
R33	5	95
K93	78	22
L96	81	19
M97	4	96
Y98	2	98
P99	20	80
P100	11	89
P101	28	72
Y102	2	98
Y103	84	16
L104	94	6
G105	55	45
I106	75	25
G107	90	10
Q111	95	5
Y113	95	5
I115	95	5

Table III: BIAcore analysis of Double-site CTLA4Ig mutants binding to CD86

Double-site Mutants:	% of Mutants that Bind:	% Mutant Non-binding:
L104ES25	95	5
L104EP26	93	7
L104EG27	92	8
L104EK28	70	30

L104EA29	94	6
L104ET30	95	5
L104EK93	82	18
L104EG105	94	6
L104EI106	92	8
L104EG107	90	10

Table IV: BIAcore analysis of Triple-site CTLA4Ig mutants binding to CD86

Triple-site mutants:	% of Mutants that Bind:	% Mutant Non-binding:
L104EA29YS25	95	5
L104EA29YP26	93	7
L104EA29YG27	95	5
L104EA29YK28	81	19
L104EA29YT30	92	8
L104EA29YL98	96	4

5

Flow Cytometry:

10 Murine MAb L307.4 (anti-CD80) was purchased from Becton Dickinson (San Jose, California) and IT2.2 (anti-B7-0[also known as CD86]), from Pharmingen (San Diego, California). For immunostaining, CD80 and/or CD86 +CHO cells were removed from their culture vessels by incubation in phosphate-buffered saline containing 10mM EDTA. CHO cells ($1-10 \times 10^5$) were first incubated with MAbs or immunoglobulin fusion proteins in DMEM containing 10% fetal bovine serum (FBS), then washed and incubated with fluorescein isothiocyanate-conjugated goat anti-mouse or anti-human immunoglobulin second step reagents (Tago, Burlingame, California). Cells were given a final wash and analyzed on a FACScan (Becton Dickinson).

FACS analysis (Figure 29 A and B) of CTLA4Ig and mutant molecules binding to stably transfected CD80+ and CD86+CHO cells was performed as described herein.

- 5 CD80+ and CD86+ CHO cells were incubated with increasing concentrations of CTLA4Ig, washed and bound immunoglobulin fusion protein was detected using fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin.

- 10 In Figure 29, L104EA29YIg (circles), or L104EIg (triangle) CHO cells (1.5×10^5) were incubated with the indicated concentrations of CTLA4Ig (closed square), L104EA29YIg (circles), or L104EIg (triangle) for 2 hr. at 23 °C, washed, and incubated with fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin antibody. Binding on a total of 5,000 viable cells was analyzed (single determination) on a FACScan, and mean fluorescence intensity (MFI) was determined from data histograms using PC-LYSYS.
- 15 Data have been corrected for background fluorescence measured on cells incubated with second step reagent only (MFI = 7). Control L6 MAb (80 µg/ml) gave MFI < 30. This is representative of four independent experiments.

- 20 The binding of L104EG105FIg, L104EIg, and CTLA4Ig (Figure 35 A and B), or the binding of L104ES25RIg, L104EIg, and CTLA4Ig (Figure 37 A and B) to CHO cells stably transfected with human CD80 or CD86 were also compared. The transfected CHO cells were incubated with increasing concentrations of proteins for 2 hours at 23 degrees C, washed and incubated with fluorescein-conjugated goat anti-human IgG. Binding on a total of 5,000 viable cells was analyzed on a FACScan, and mean fluorescence intensity
- 25 determined from data histograms using PC-LYSYS.

Functional Assays:

- 30 Human CD4⁺T cells were isolated by immunomagnetic negative selection (Linsley et al., 1992 *J. Exp. Med.* 176:1595-1604).

Inhibition of PMA plus CD80-CHO or CD86-CHO T cell stimulation (Figure 30 A and B) was performed, comparing CTLA4Ig and L104EA29YIg. CD4⁺T cells (8×10^4 /well) were cultured in the presence of 1 nM PMA with or without irradiated CHO cell stimulators. Proliferative responses were measured by the addition of 1 μ Ci/well of [³H]thymidine during the final 7 hr. of a 72 hr. culture.

The effect of L104SIg, L104EIg, and CTLA4 were also compared. The effects of increasing concentrations of CTLA4Ig, L104EIg, L104SIg, or isotype matched negative control were measured on stably transfected CD80-CHO (Figure 34 A) and CD86-CHO cell costimulation of PMA-stimulated peripheral blood CD4⁺ T cells. T cells (5.0×10^4 cells/well) stimulators (Figure 34 B). Proliferation was measure by the addition of 1.0 micro Ci/well of ³H-thymidine during the final 7 hours of a 72 hour culture. The symbols for L104EA29YIg (open circle), CTLA4Ig (closed square), and control (open triangle) represent means (\pm standard deviations) of triplicate determinations.

Figures 31 A and B, and 26 A and B show inhibition of allostimulated human T cells prepared above, and allostimulated with a human B LCL line called PM. T cells at 3.0×10^4 /well and PM at 8.0×10^3 /well. Primary allostimulation occurred for 6 days then the cells were pulsed with ³H-thymidine for 7 hours before incorporation of radiolabel was determined. Secondary allostimulation was performed as follows. Seven day primary allostimulated T cells were harvested over LSM (Ficol) and rested for 24 hours. T cells then restimulated (secondary) by adding PM in same ratio as above. Stimulation occurred for 3 days, then the cells were pulsed with radiolabel and harvested as above. To measure cytokine production (Figures 32 A and B), duplicate secondary allostimulation plates were set up. After 3 days, culture media was assayed using Biosource kits using conditions recommended by manufacturer.

The inhibition of proliferation of primary allostimulated Tcells was also performed using L104EIg and L104EG105FIg (Figure 36). Purified CD4⁺ cells (5.0×10^4 cells/well) were incubated with an irradiated LCL allogeneic B cell line (1.0×10^4 cells/well) in the presence of increasing concentrations of CTLA4Ig, L104EIg, L104EG105FIg, or isotype

negative control. Proliferation was measured by ^3H -thymidine incorporation over the final 7 hours of a 6 day assay. Symbols for CTLA4Ig (closed diamond), control (closed square), L104EIg (open triangle), and L104EG105FIg (open square) represent means (\pm standard deviations) of triplicate determinations.

Monkey MLR (Figure 33). PBMC'S from 2 monkeys purified over LSM and mixed (3.5×10^4 cells/well from each monkey) with 2 $\mu\text{g}/\text{ml}$ PHA. Stimulated 3 days then pulsed with radiolabel 16 hours before harvesting.

10 EXAMPLE 10

The following provides a description of the methods used to generate the soluble CTLA4 mutants, using phage display techniques.

15 High Avidity Mutagenesis of CTLA4Ig through Phage Display System

Recombinant phage display technology was performed to select CTLA4Ig mutant molecules which bind to CD80 and/or CD86. This method was used to screen millions of CTLA4 mutants, express them on the surface of phage particles, and select strong
20 CD86Ig binders by a panning procedure. The pCANTAB 5E phagemid system (Pharmacia Biotech) was used in this study.

25 Expression of wild type CTLA4X (extracellular domain) in pCANTAB 5E phage display system

Cloning:

The region encoding the complete CTLA4 extracellular domain was cloned into pCANTAB 5E vector to create a fusion protein with Gene III (phage coat protein). The pCANTAB 5E - CTLA4 X was transformed into TG1 E. coli bacterial cells.

Phage rescue:

5 The bacteria containing the vector encoding the wild-type CTLA4-Gene III fusion protein was infected with helper phage. Phage particles were assembled in bacteria and released into the media. Each phage particle was expressed approximately 1 CTLA4-Gene III fusion protein on the surface. 0.1 ml of bacterial culture was added to 5 ml of 2x TY AG medium, the bacteria were grown at 30 degrees C for 2 hours. 0.25 ml of M13K07 helper phage (5×10^{10} /ml) was added, the cells were shaken at 37 degrees C, 250 rpm for 1 hr. The cells 10 were spun down and the supernatant was discarded. The cell pellet was resuspended in 5 ml 2 x YT AK medium, shaken at 250 rpm, 37 degrees C overnight. The cells were spun down and saved as the supernatant as phage stock.

Mutagenesis of CTLA4X

15 Mutation sites occurred in a region encompassed by tyrosine at position +23 through threonine at position +30 (e.g., Y23-T30). All amino acids residues within the Y23-T30 were mutated to each of the 20 amino acids, yielding a total diversity of nucleotide sequences of about 2.8×10^{14} .

Mutagenesis and preparation of phage display library

From tyrosine +23 to threonine +30, a pool of degenerate forward primers were generated having the following sequence:

25 5' CGA GGC ATC GCT AGC TTT GTG TGT GAG XXK XXK XXK XXK XXK XXK
XXK XXK GAG GTC CGG GTG ACA GT 3'

Where X = any of the four nucleotides A, T, G, or C and K = either T, G, or C

Each primer contained a Nhe I restriction enzyme cut site.

30 The reverse primer had the following sequence:

5' GGT TGC CGC ACA GAC TTC GGT CAC CTG GCT GTC AGC CTG CCG AAG
CAC TGT CAC CCG GA 3'

This primer contained a BstE II restriction enzyme cut site.

By PCR, mutagenic inserts were prepared using the degenerate primers and the standard reverse primer. These inserts were digested with Nhe I and BstE II and ligated into pCANTAB-CX2 phagemid digested with the same enzymes and transformed into TG1 cells.

Preparation of mutated B-C loop-pCANTAB-CX2 library:

45 electroporations were performed at 1.7 KV, 300 Ω , 25 μ F, 4.5 ms with Bio-Rad Gene Pulser. The library size was 10^{12} .

Phage rescue:

Ten ml of amplified library cells were added to 40 ml 2 x YT-G-A, the cells were grown from OD 0.115 to 0.332 at 37 degrees C. Total cells = $0.332 \times 10^9 \times 50 = 1.66 \times 10^{10}$ M13K07 helper phage ($20 \times 1.66 \times 10^{10} = 3.32 \times 10^{11}$) were added to the culture and incubated at 30 degrees C with shaking for 1.5 hours. The culture was spun down and the supernatant removed. The cells were resuspended into 50 ml 2x TY A-K medium and incubated at 30 degrees C with shaking at 250 rpm overnight. The cells were spun down and the supernatant was saved. The phage was PEG precipitated from the supernatant. The precipitated phage was used for the first round panning procedure.

Panning:

The phage particles expressing a mutant CTLA4-Gene III fusion protein were allowed to bind to CD86 immobilized on plastic. The strong binders were preferentially selected and enriched. A 96-well plate was coated with CD86Ig at 4 micro grams/well at 4 degrees C overnight. The wells were blocked with PBS-milk for 2 hours. The phage were mixed with equal volumes of PBS-milk and incubated at room temperature for 30 minutes. 200 micro liters of phage mixture was added to each well and incubated for 2 hours. The wells were washed with PBS tween and eluted with glycine buffer pH 2.2. The eluted phage were collected, used to reinfect TG1 cells, rescued and panned several times.

Panning results:

5	1 st round:	Input phage	6×10^{11}
		Wash (x7)	2.8×10^8
		Elute	3×10^8
10	2 nd round:	Input phage	4.8×10^{11}
		Wash 10	7×10^7
		Elute	4.5×10^7
15	3 rd round:	Input phage	1×10^{12}
		Wash 12	2×10^7
		Elute	3×10^7
20	4 th round:	Input phage	4×10^{11}
		Wash 15	2×10^7
		Elute	1×10^9
25	5 th round:	Input phage	8×10^{11}
		Wash 35	7×10^7
		Elute	5×10^9

Five mutants were enriched through these 5 rounds of panning.

25	Mut 9	F-E-P-K-R-G-V-Q
	Mut 19	W-D-Q-Y-T-G-Y-G
	Mut 71	W-D-A-Y-R-N-Q-Q
	Mut 45	Y-D-H-P-Y-D-G-Q
30	Mut 4	W-D-Q-H-V-S-R-R
	CTLA4	Y-A-S-P-G-K-A-T

What is claimed is:

1. A soluble CTLA4 mutant molecule having the extracellular domain of CTLA4 which binds CD80 or CD86.
2. The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 has one or more mutations in a region S25-R33 of CTLA4, and wherein the mutation is a substitution of any amino acid of serine at +25 through lysine at +28 with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.
3. The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 has one or more mutations in a region S25-R33 of CTLA4, and wherein the mutation is a substitution of alanine at position +29 with any of arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, or valine.
4. The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 has one or more mutations in a region S25-R33 of CTLA4, and wherein the mutation is a substitution of any amino acid of threonine at +30 through arginine at +33 with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.
5. The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 has one or more mutations in a region E95-G107 of CTLA4, and wherein the mutation is a substitution of any amino acid of

glutamic acid at +95 through lysine at +96 with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

5

10

6. The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 has one or more mutations in a region E95-G107 of CTLA4, and wherein the mutation is a substitution of any amino acid of methionine at +97 through tyrosine at +103 with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

15

7. The soluble CTLA4 mutant molecule of claim 5, wherein the mutation is a substitution of tyrosine at position +103 with a different amino acid selected from a group consisting of arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

20

25

8. The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 has one or more mutations in a region E95-G107 of CTLA4, and wherein the mutation is a substitution of any amino acid of leucine at +104 through glycine at +107 with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

30

9. The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 has one or more mutations in a region beginning with

asparagine at position +108 and ending at isoleucine at position +115 (N108-I115).

- 5 10. The soluble CTLA4 mutant molecule of claim 9, wherein the mutation is a substitution of any of the amino acids in N108-I115 with a different amino acid selected from a group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.
- 10 11. The soluble CTLA4 mutant molecule of claim 1 further comprising an amino acid sequence which alters the solubility, affinity or valency of the soluble CTLA4 mutant molecule for binding to CD80 or CD86.
- 15 12. The soluble CTLA4 mutant molecule of claim 14, wherein the amino acid sequence comprises a human immunoglobulin constant region.
- 20 13. A nucleic acid molecule comprising a nucleotide sequence encoding the amino acid sequence corresponding to the soluble CTLA4 mutant molecule of claim 1.
- 25 14. A vector comprising the nucleotide sequence of claim 13.
15. A host vector system comprising the vector of claim 14 in a suitable host cell.
- 25 16. The host vector system of claim 15, wherein the suitable host cell is a prokaryotic cell or a eukaryotic cell.
- 30 17. A method for producing a soluble CTLA mutant protein comprising growing the host vector system of claim 16 so as to produce the protein in the host cell and recovering the protein so produced.

18. A soluble CTLA mutant protein produced by the method of claim 17.

19. A method for regulating a T cell interaction with a CD80 and/or CD86 positive cell comprising contacting the CD80 and/or CD86 positive cell with the soluble CTLA4 mutant molecule of claim 1 so as to regulate the T cell interaction.

20. The method of claim 19, wherein the soluble CTLA4 mutant molecule is any of L104EA29L, L104EA29T, or L104EA29W.

21. The method of claim 19, wherein the CD80 and/or CD86 positive cell is an antigen presenting cell.

22. The method of claim 19, wherein the interaction of the CTLA4-positive T cells with the CD80 and CD86 positive cells is inhibited.

23. A method for treating immunoproliferative diseases mediated by T cell interactions with B7 positive cells comprising administering to a subject the soluble CTLA4 mutant molecule of claim 1, in an amount effective to regulate T cell interactions with said B7 positive cells.

24. The method of claim 23, wherein said T cell interactions are inhibited.

25. The method of claim 23, wherein the immunoproliferative disease is graft versus host disease.

SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

5 ABSTRACT OF THE INVENTION

The present invention provides soluble CTLA4 mutant molecules which bind CD80 and/or CD86 antigen.

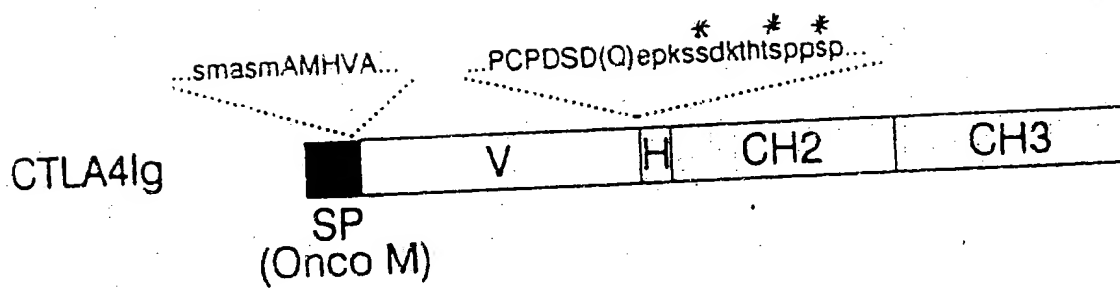


Figure 1

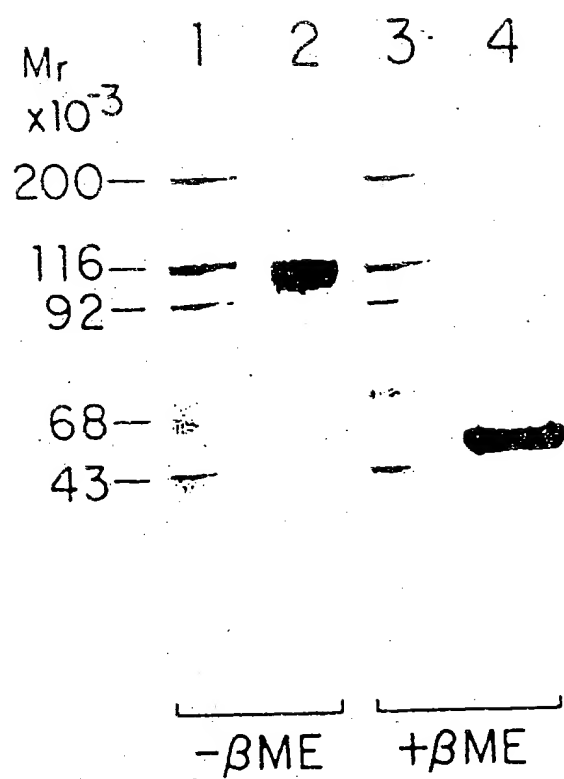


Figure 2

-26

M G V L L T Q R T L L S L V L 45
ATG GGT GTA CTG CTC ACA CAG AGG ACG CTG CTC ACT CTG GTC CTT

A L L F P S M A S M A H H V A 90
GCA CTC CTG TTT CCA AGC ATG GCG AGC ATG GCA ATG CAC GTG GCC

Q P A V V L A S S R G I A S F 135
CAG CCT GCT GTG GTA CTG GCC AGC AGC CGA GGC ATC GCC AGC TTT

V C E Y A S P G K A T E V R V 180
GTG TGT GAG TAT GCA TCT CCA GGC AAA GCC ACT GAG GTC CGG GTG

T V L R Q A D S Q V T E V C A 225
ACA GTG CTT CGG CAG GCT GAC AGC CAG GTG ACT GAA GTC TGT GCG

A T Y M M G N E L T F L D D S 270
GCA ACC TAC ATG ATG GGG AAT GAG TTG ACC TTC CTA GAT GAT TCC

I C T G T S S G N Q V N L T I 315
ATC TGC ACG GGC ACC TCC ACT GGA AAT CAA GTG AAC CTC ACT ATC

Q G L R A M D T G L Y I C K V 360
CAA GGA CTG AGG GCC ATG GAC ACG GGA CTC TAC ATC TGC AAG GTG

E L M Y P P P Y Y L G I G N G 405
GAG CTC ATG TAC CCA CCG CCA TAC TAC CTG GGC ATA GGC AAC GGA

T Q I Y V I D P E P C P D S D 450
ACC CAG ATT TAT GTA ATT GAT CCA GAA CCG TGC CCA GAT TCT GAC

F L L W I L A A V S S G L F F 495
TTC CTC CTC TGG ATC CTT GCA GCA GTT AGT TCG GGG TTG TTT TTT

Y S F L L T A V S L S K M L K 540
TAT AGC TTT CTC CTC ACA GCT GTT TCT TTG AGC AAA ATG CTA AAG

K R S P L T T G V Y V K M P P 585
AAA AGA AGC CCT CTT ACA ACA GGG GTC TAT GTG AAA ATG CCC CCA

T E P E C E K Q F Q P Y F I P 630
ACA GAG CCA GAA TGT GAA AAG CAA TTT CAG CCT TAT TTT ATT CCC

I N
ATC AAT

636

Figure 3

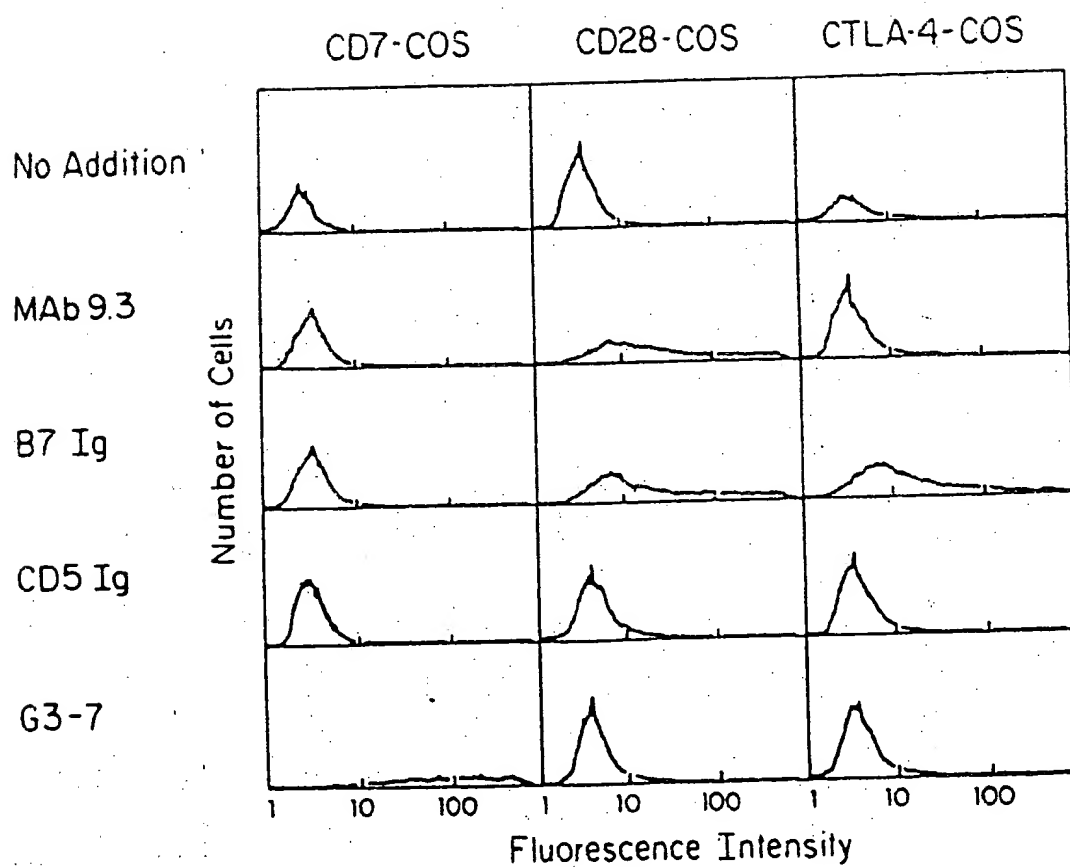


Figure 4

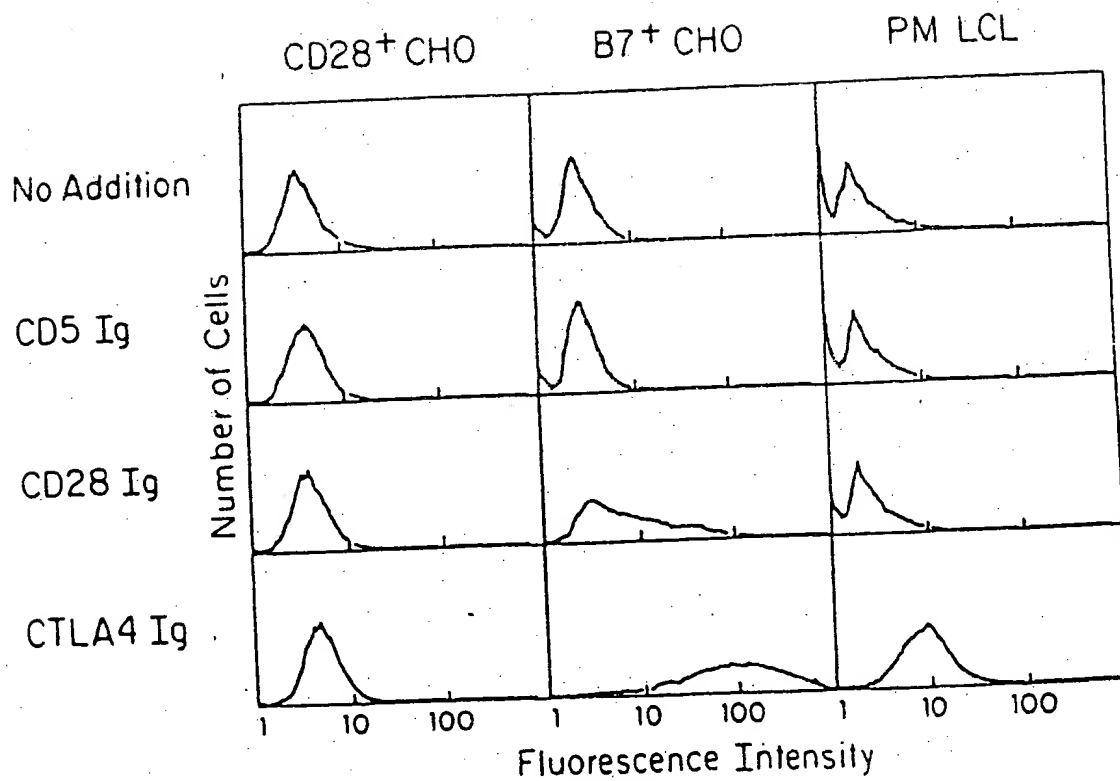


Figure 5

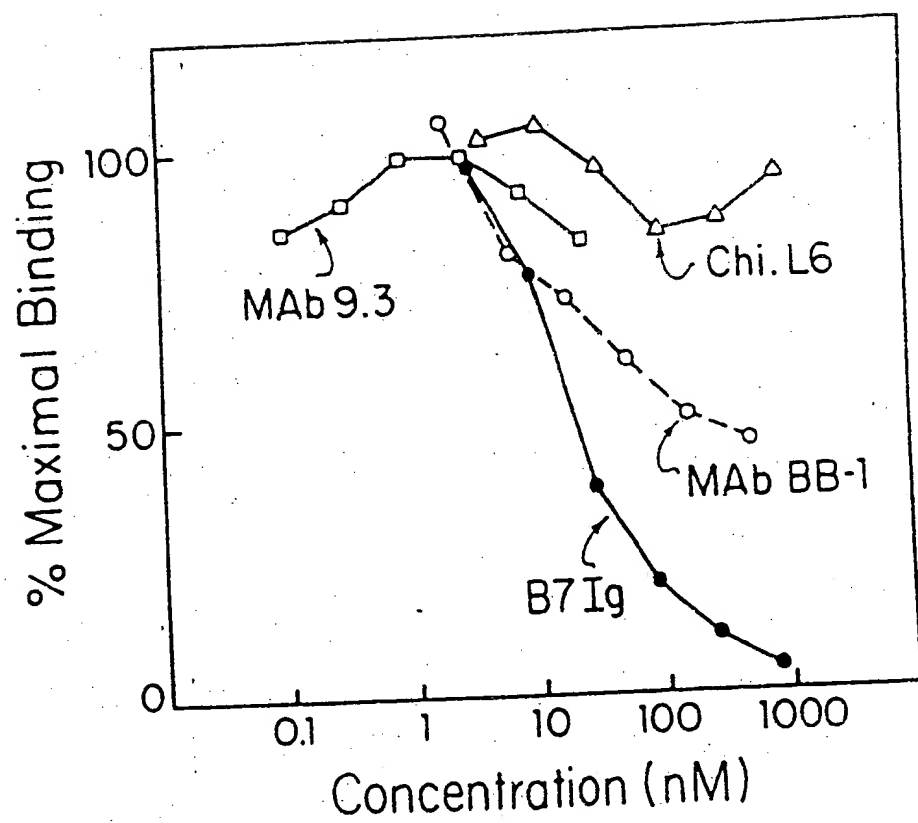


Figure 6

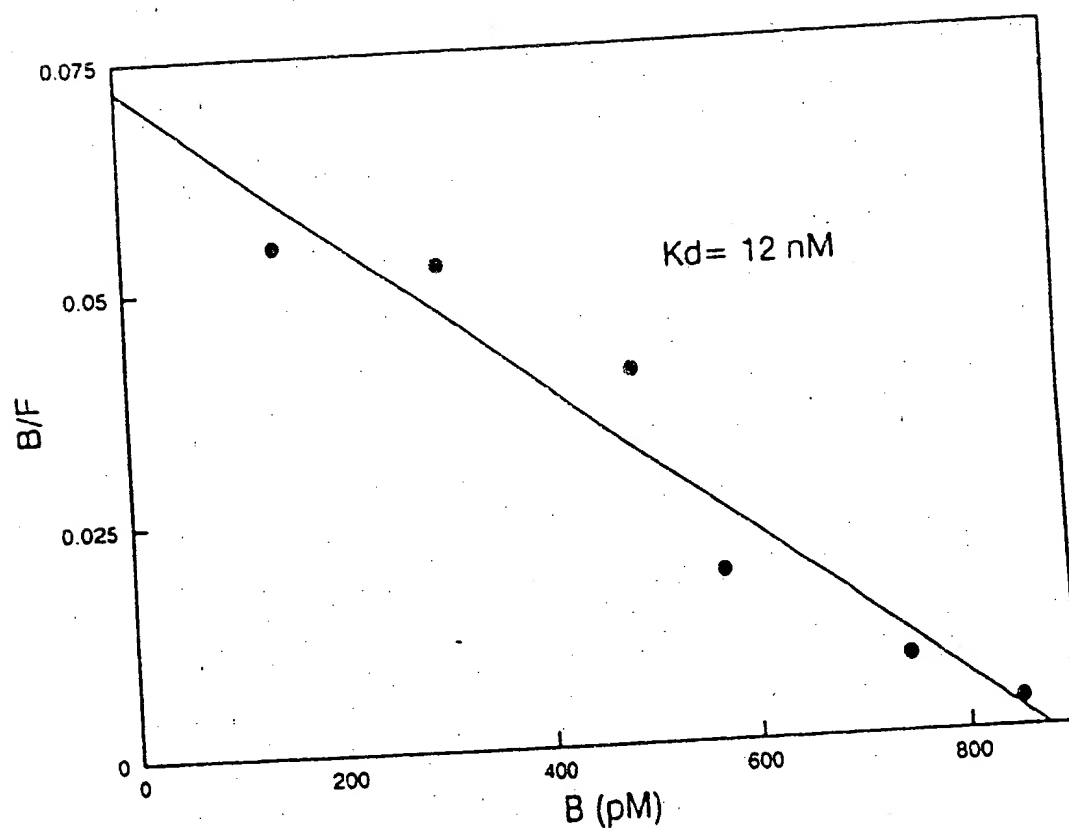


Figure 7

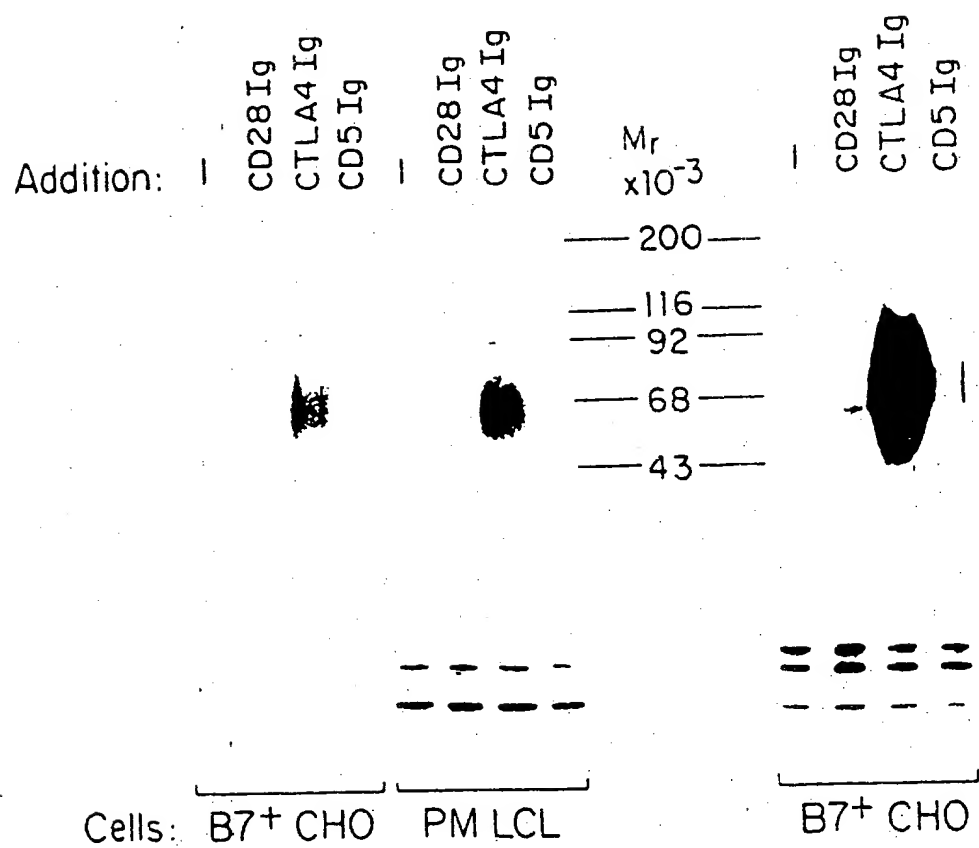


Figure 8

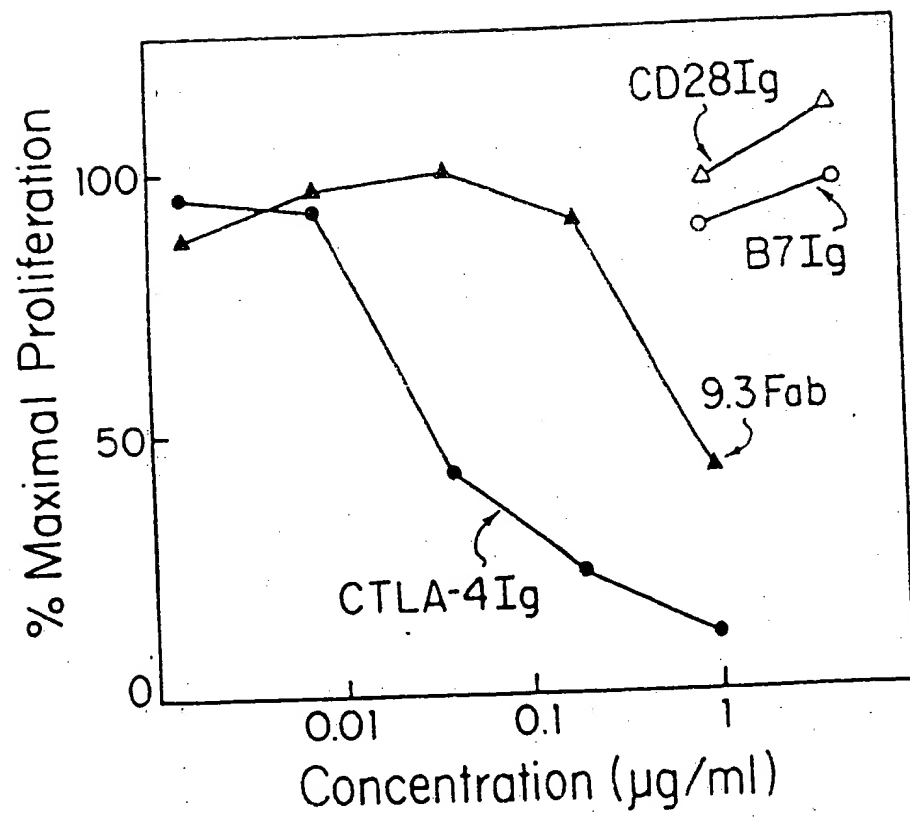


Figure 9

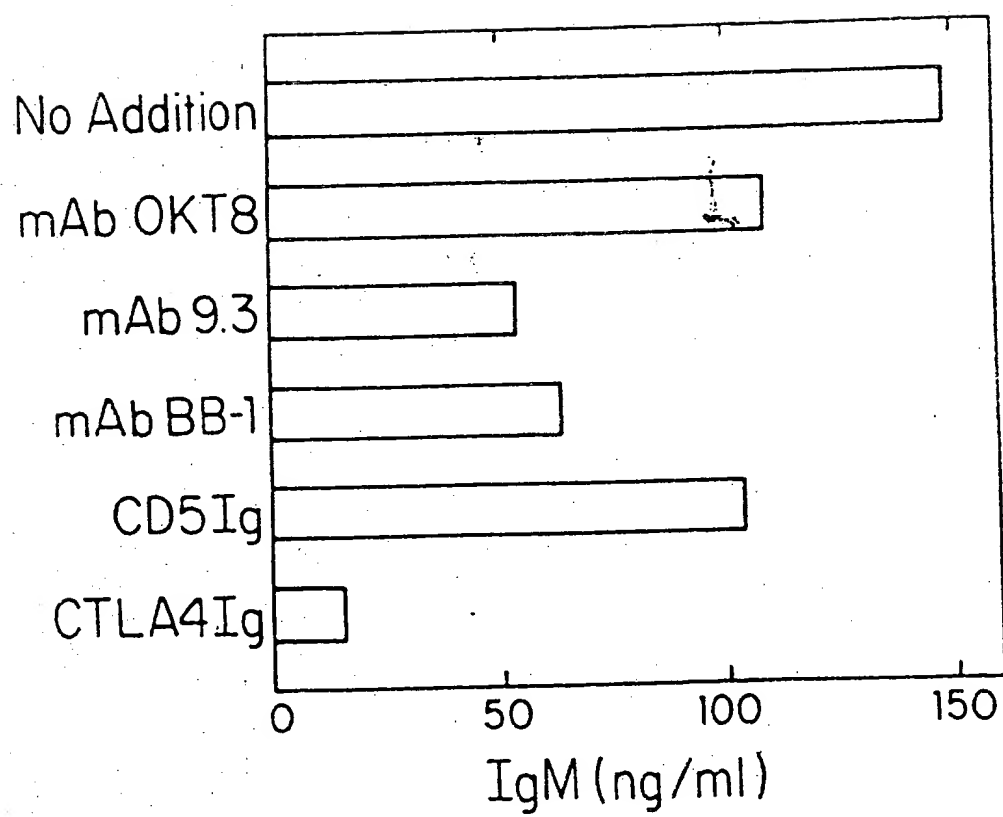


Figure 10

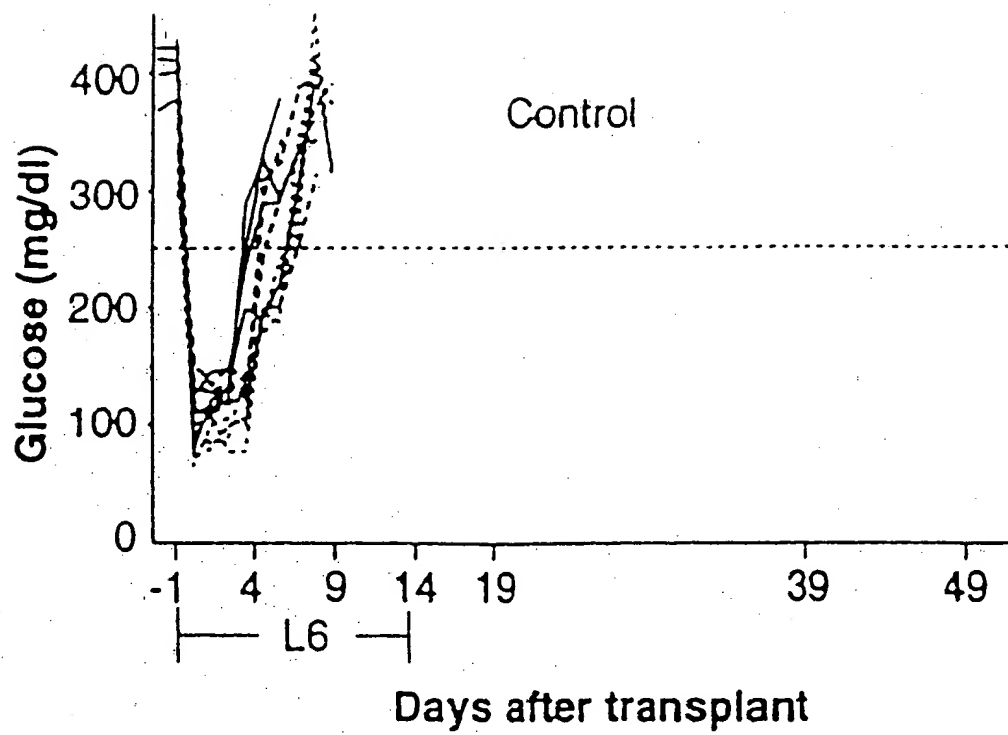


Figure 11A

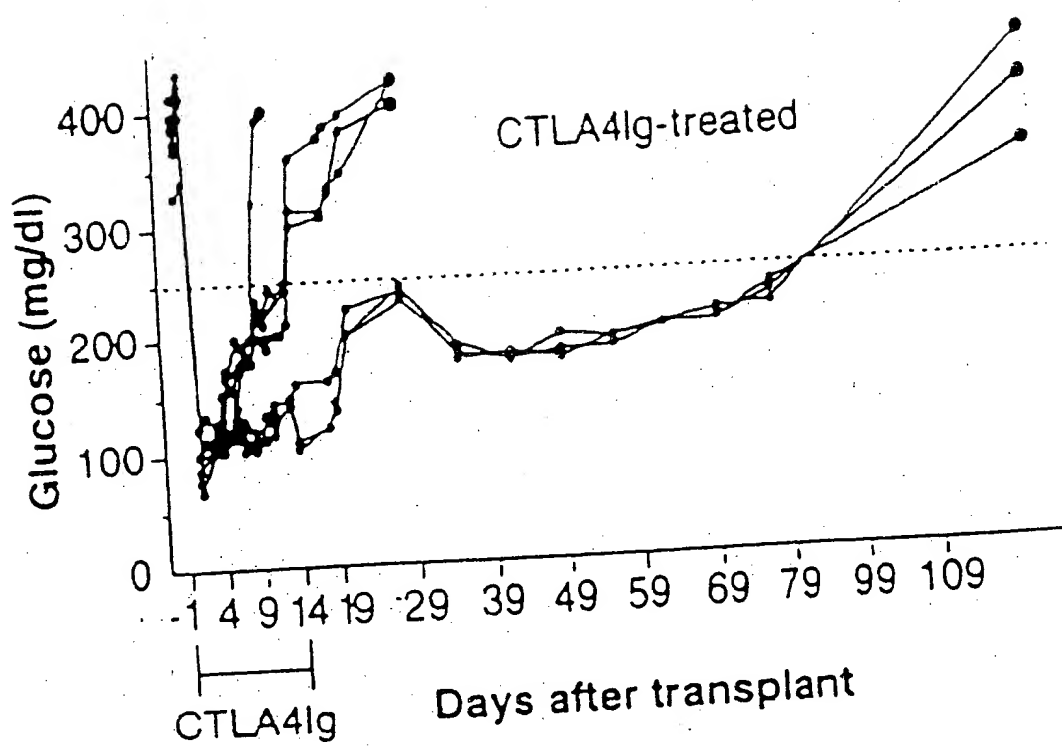


Figure 11B

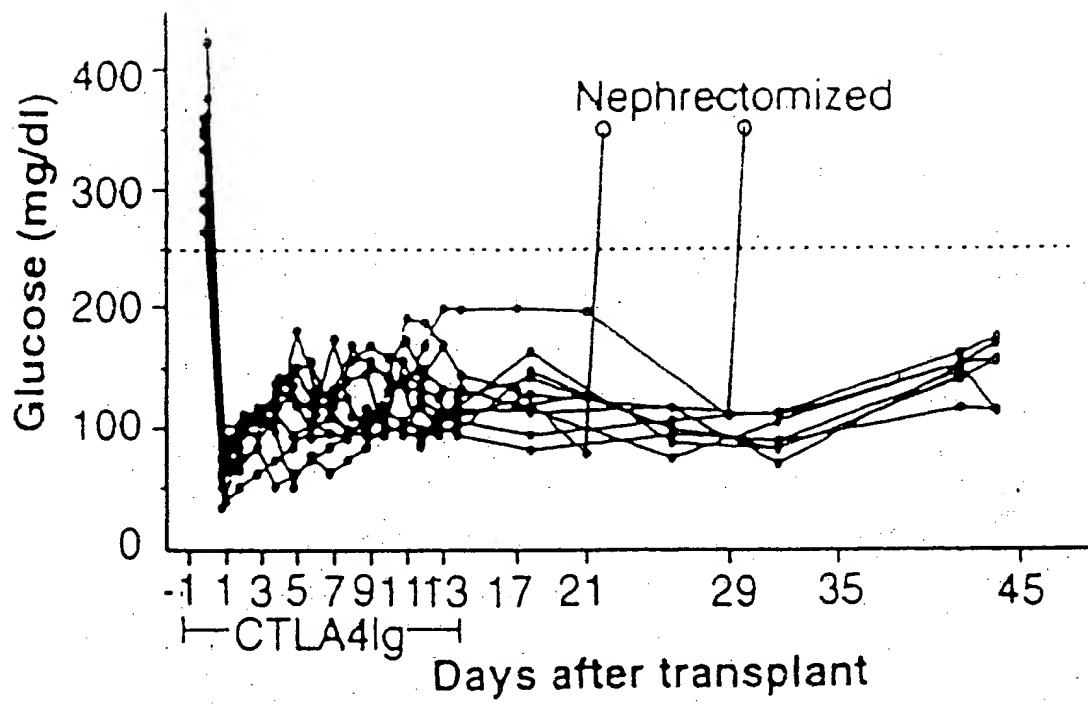


Figure 11C

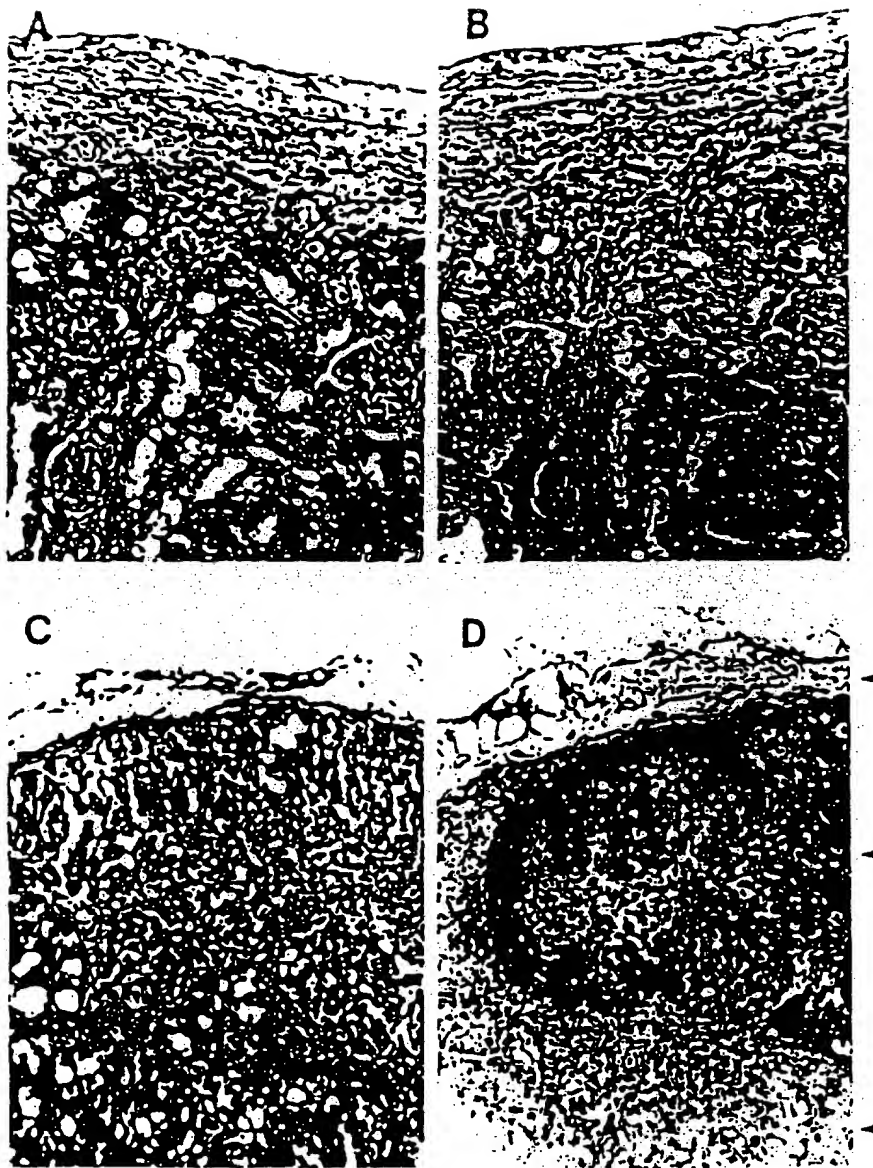


Figure 12

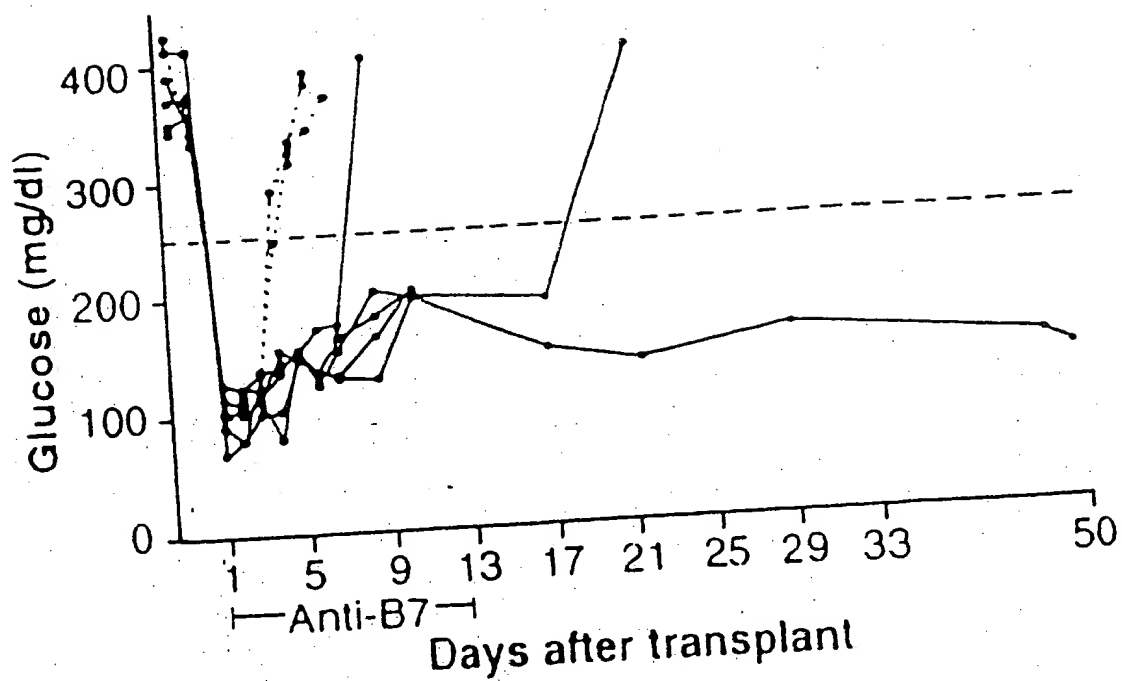


Figure 13

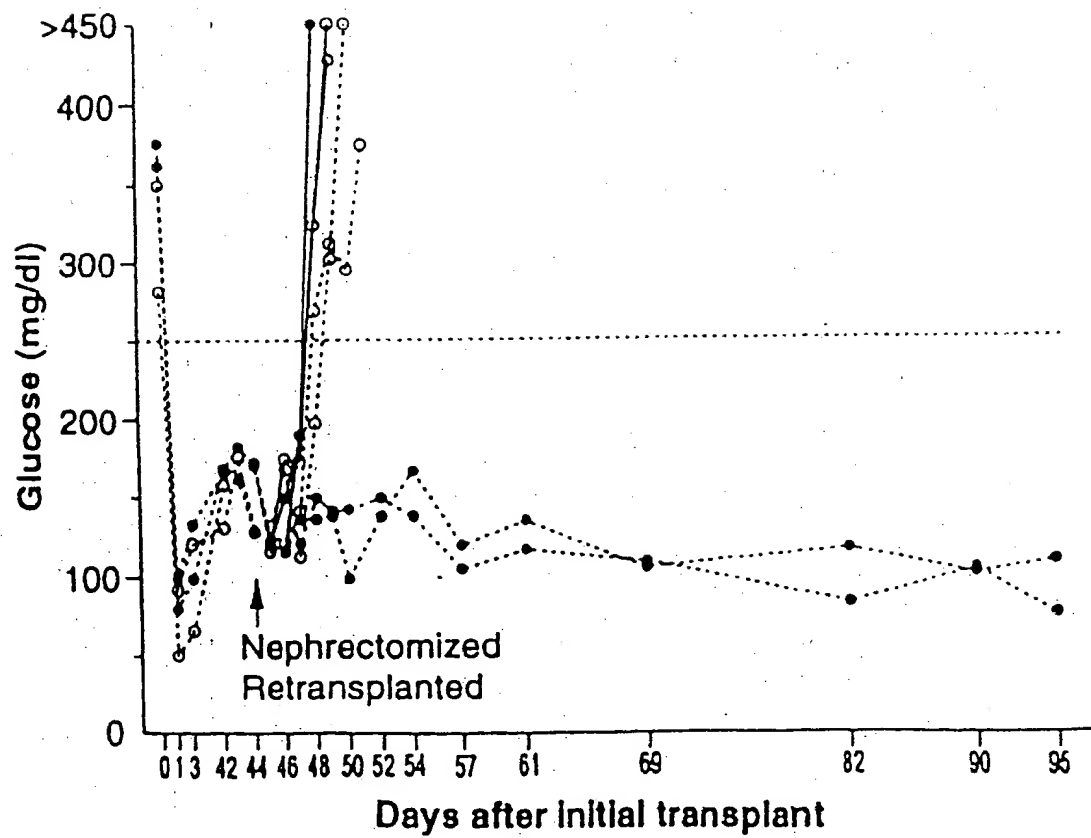


Figure 14

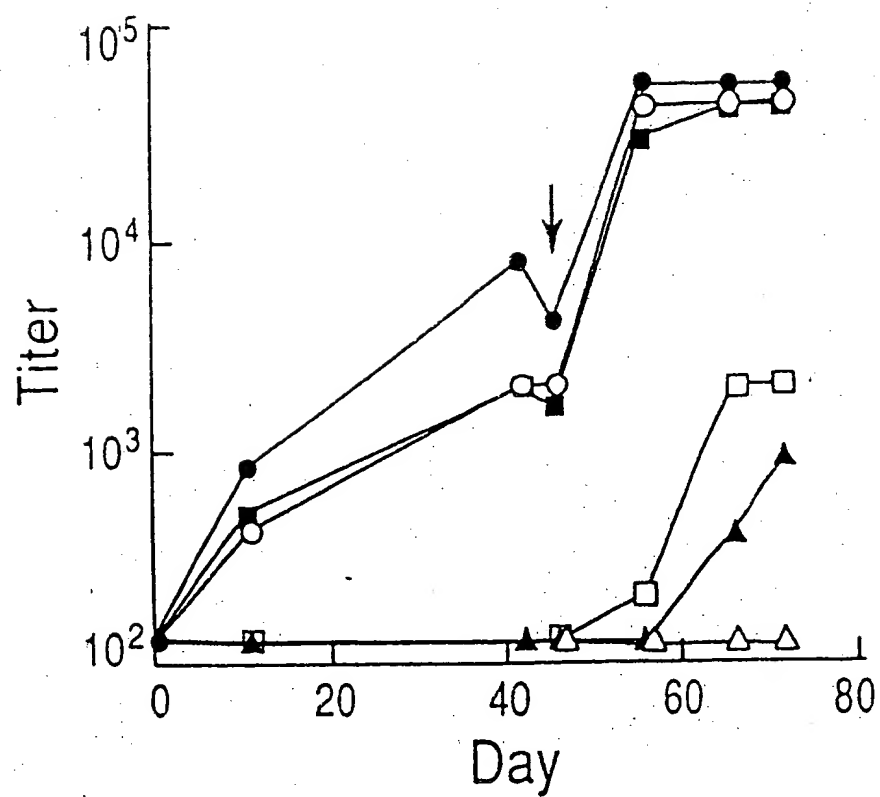


Figure 15

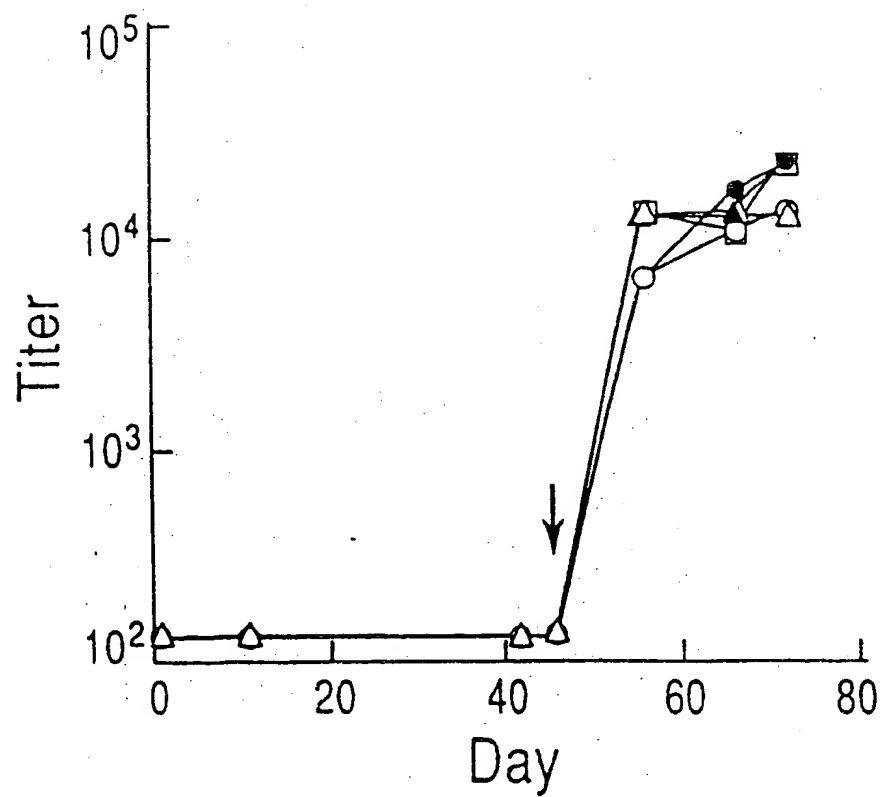


Figure 16

CD28/CTLA-4 family

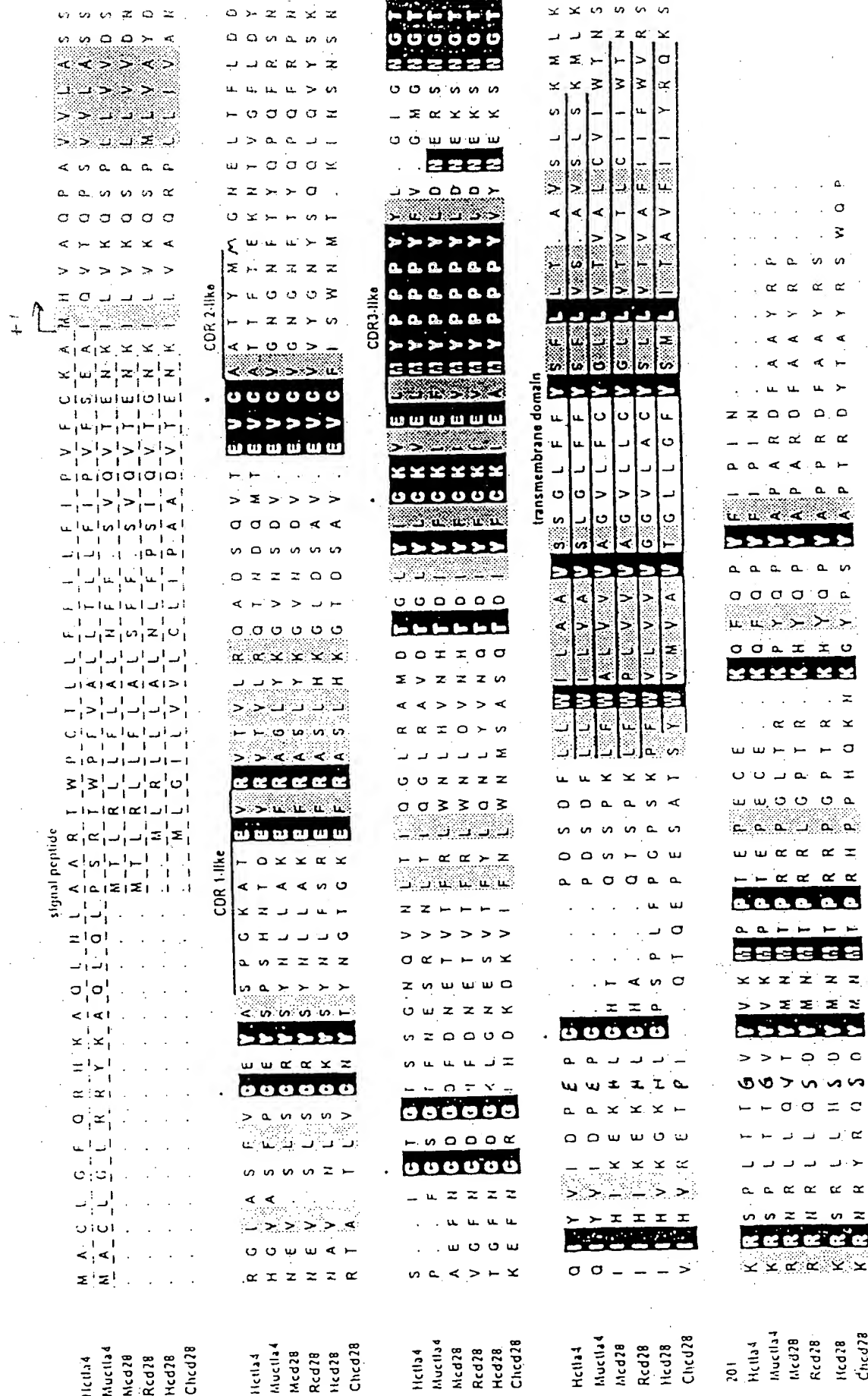


Figure 17

FIGURE 18

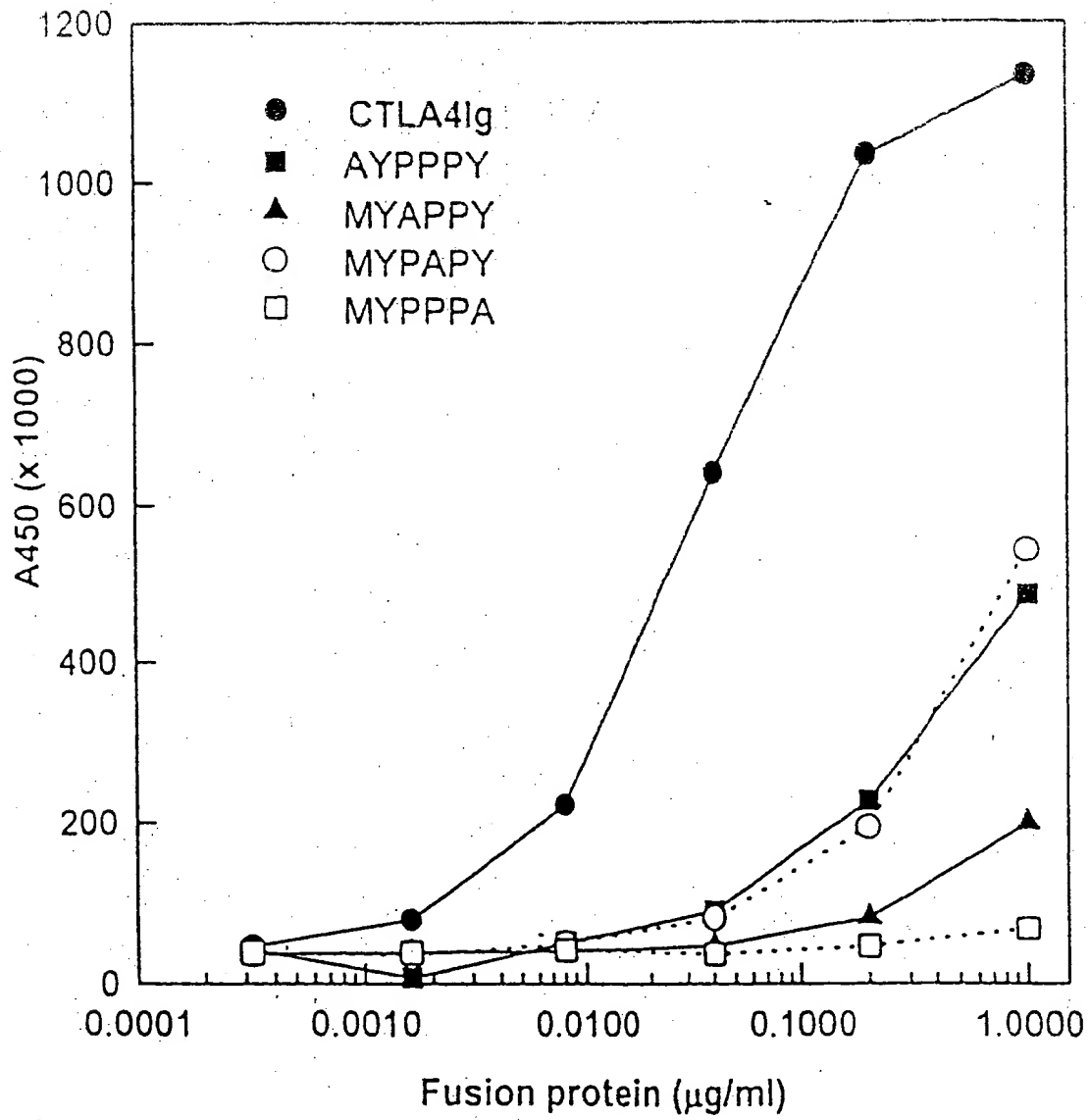


FIGURE 19

% B7
Binding Activity

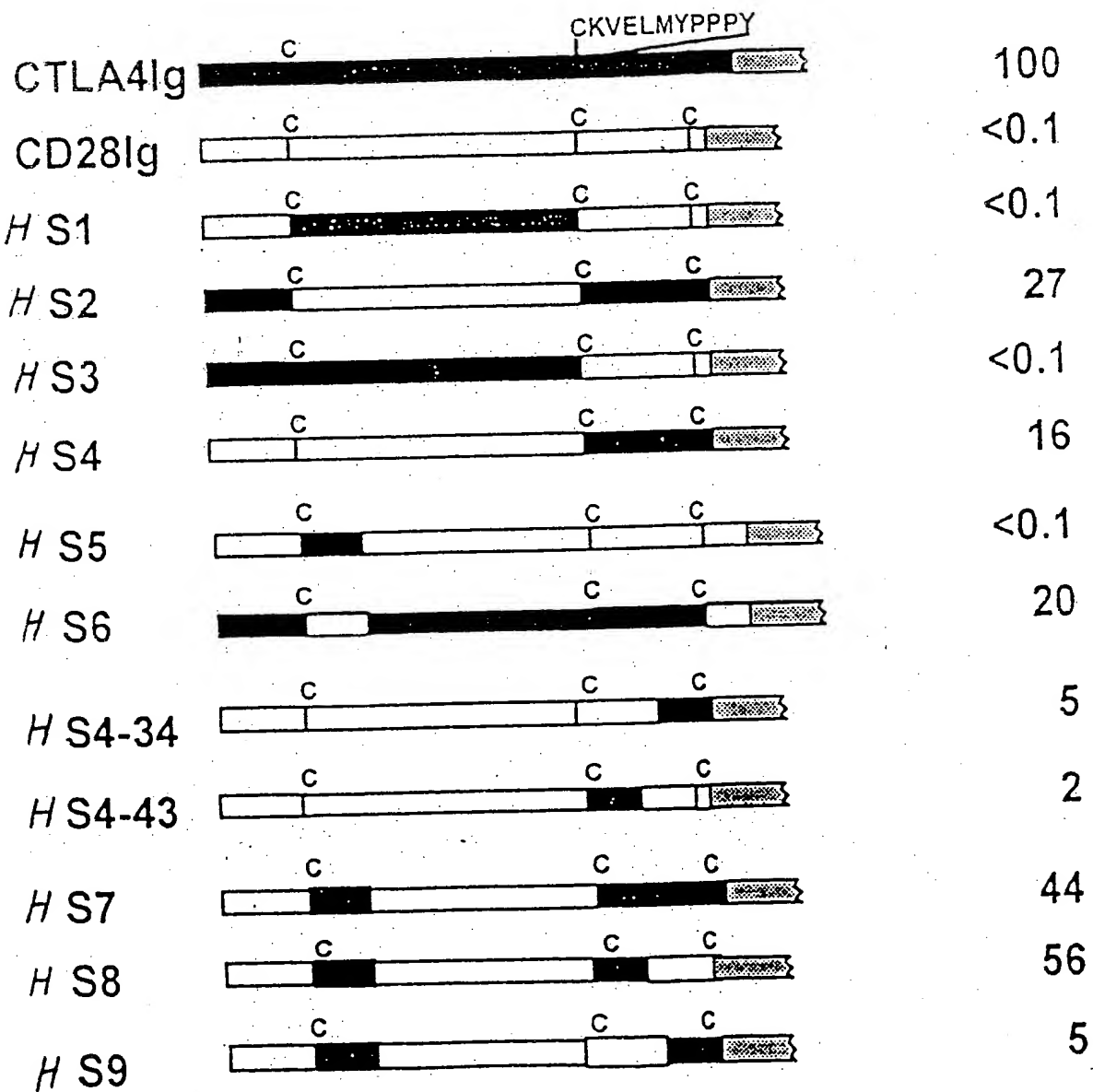


FIGURE 20(a)

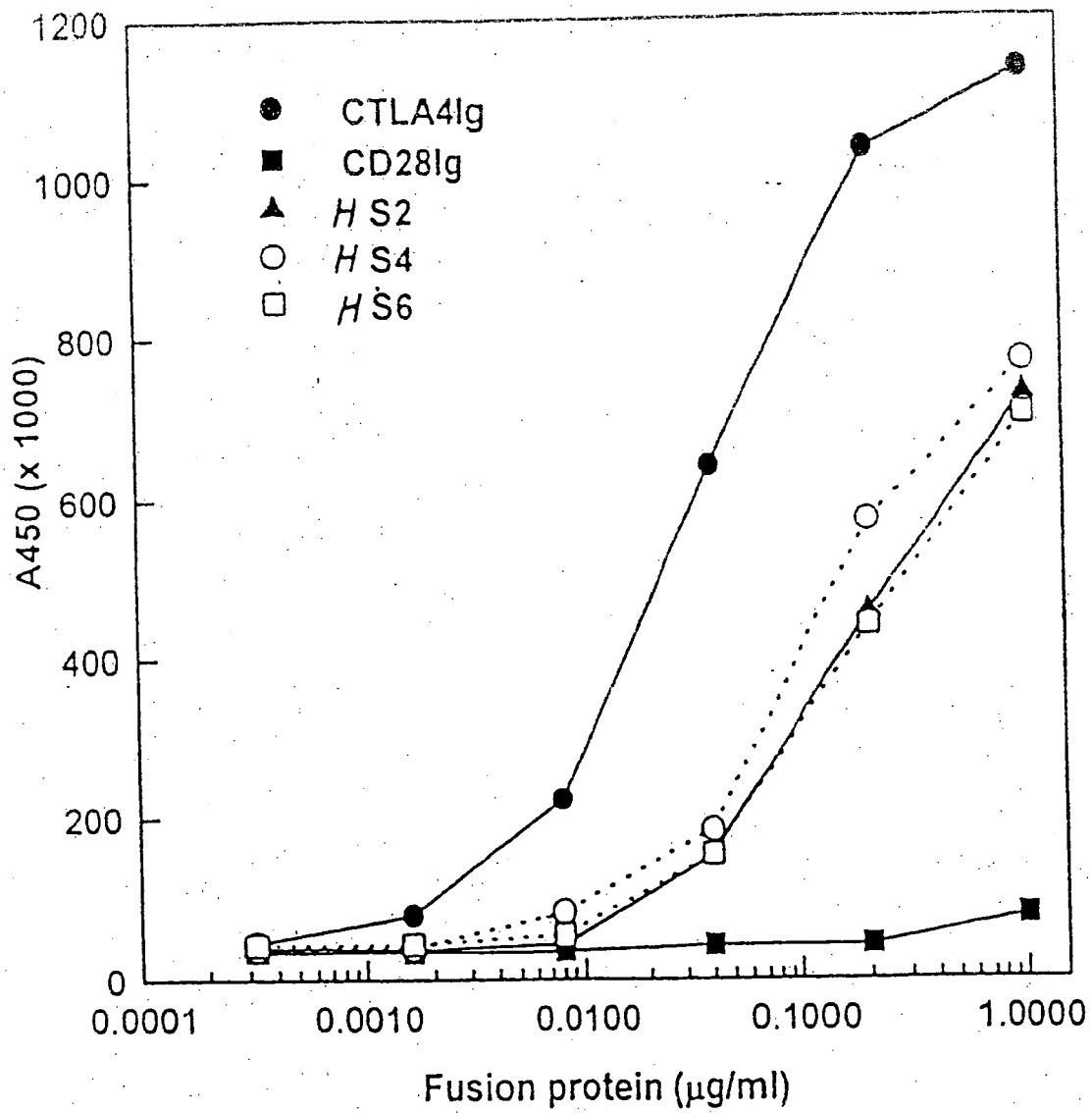


FIGURE 20(b)

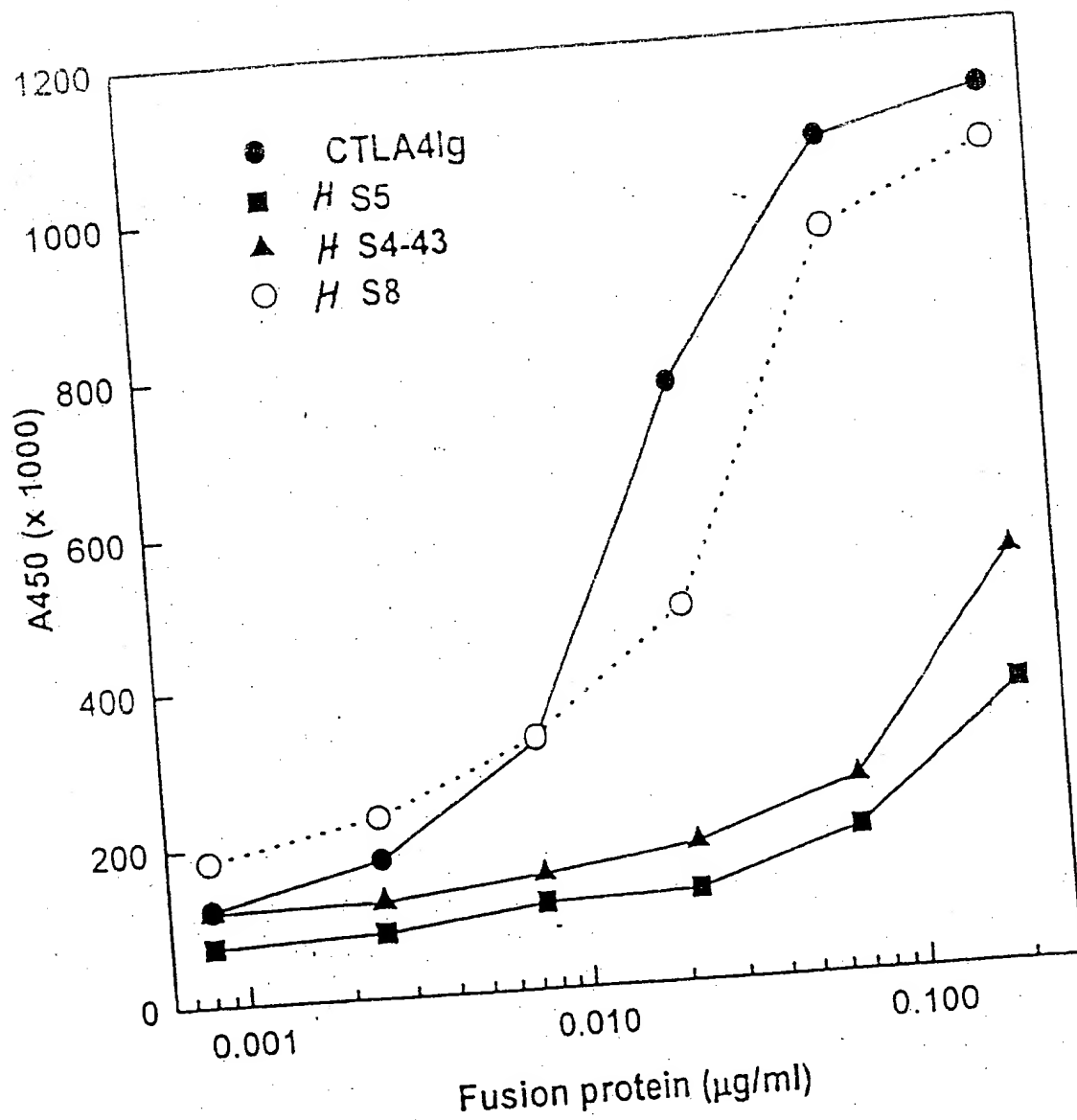
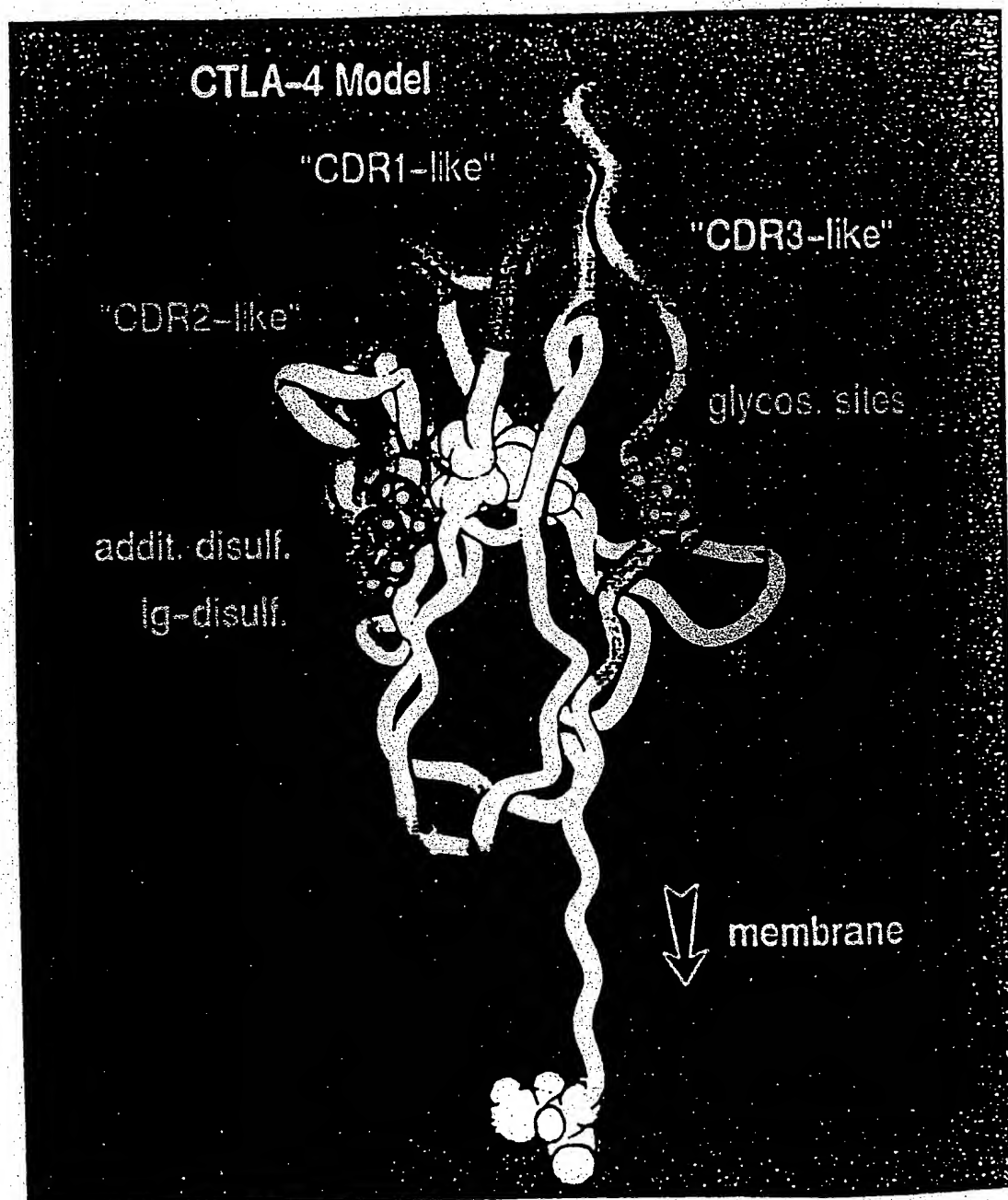


FIGURE 21



amino acid
-2.6

nucleotide
-78

ATGGGTGTACTGCTCAGACAGAGAGCGGTGCTCACTCTGGTCTTCCACTCTCTTTTCCA
(H) G-V-L-L-T-Q-R-T-L-L-S-L-V-L-A-L-L-P-P-
AGCATGGCAGCATGGCATGCTACGTCGCGCCAGCCTGCTGTGCTACTGGCCAGCAGCCGA
S-H-A-S-H-A-M-H-V-A-Q-P-A-V-V-L-A-S-S-R-
GGCATGGCAGCTTTTGTGTGAGTATGATCTCCAGCCAAAGCCACTGAGGTCCGGTG
G-I-A-S-F-V-C-E-Y-A-S-P-G-K-A-T-E-V-R-V-
ACAGTCTTGGCAGCCTGACAGCCAGGTGACTGAAGTCTGTGCGGCAACCTACATGATG
T-V-L-R-Q-A-D-S-Q-V-T-E-V-C-A-A-T-Y-H-H-
GGGAATGAATTGACCTTCTAGATGATTCCATCTCCAGCGCCAGCTCCAGTGGAAATCA
G-H-E-L-T-F-L-D-D-S-I-C-T-G-T-S-S-Q-N-Q-
GTGAACCTCACTATCCAAAGACTGAGGCGCATGGACACGGGACTCTACATCTCCAGGTG
V-N-L-T-I-Q-G-L-R-A-H-D-T-G-L-Y-I-C-K-V-
GAGCTCATGTACCCAGCCGCTACTACTCTGGCATAGCCAGCGAACCCAGATTATGTA
E-L-H-Y-P-P-P-Y-Y-L-G-I-G-N-G-I-Q-I-Y-V-
ATTGATCCAGAACCGTGGCCAGATTCTGATCAGGAGCCAAATCTTCTGACAAACTCAC
I-D-P-E-P-C-P-D-S-D-Q-E-P-K-S-S-D-K-T-H-
ACATCCCCAGCCTCCCCAGCAGCTGAAGTCTCTGGTGGATGCTCAGTCTTCTCTCCCC
T-S-P-P-S-P-A-P-E-L-L-O-Q-S-S-V-F-U-P-P-
CCAAAGCCCAAGACACCCCTCATGATCTCCCGAGCCCTGAGGTACATGCGTGTGTGTG
P-K-P-K-D-T-L-H-I-S-R-T-P-E-V-T-C-V-V-V-
GACGTGAGCCAGGAGACCCCTGAGGTCAAGTCAACTGCTACGTGAGCGGCGTGGAGGTG
D-V-S-H-E-D-P-E-V-K-F-N-H-Y-V-D-Q-V-E-V-
CATAAATCCCAAGACAAAGCCCGGAGGAGCACTACAACAGCAGTACCGGGTGGTCAAG
H-N-A-K-T-K-P-R-E-Q-Y-H-S-T-Y-R-V-V-S-
GTCTCTACCCCTCTCTCAGCAGGACTGGCTGAATGGCAAGGATACAAGTGCAGGTCTCC
V-L-I-V-L-H-Q-D-N-L-N-Q-K-E-Y-K-C-X-V-S-
AACAAAGCCCTCCAGCCGCTATCCAGAAACCATCTCCAAAGCCAAAGGCGAGCCCGGA
N-K-A-L-P-A-P-I-E-K-T-I-S-X-A-K-Q-Q-P-R-
GAACCACAGGTGTACACCCCTGCCCCATCCCGGATGAGCTGACCAAGAACCAAGTCAAG
E-P-Q-V-Y-T-L-P-P-S-R-D-E-L-T-K-N-Q-V-S-
CTGACCTGCTGTGTAAGGCTTCTATCCAGCAGCATCGCCGTGGAGTGGAGAGCAAT
L-T-C-L-V-K-Q-I-Y-P-S-D-I-A-V-E-N-B-B-H-
GGGCAGCCCGAAGAACTACAAAGACCCGCTCCCGTGGTGGACTCCGAGCGCTCTTC
Q-Q-P-E-N-N-Y-K-T-T-P-P-V-L-D-S-D-Q-S-Y-
TTCTCTACAGCAAGCTCAGCCTGGACAAGGAGCAGGTGGCAGGCGGCAACCTCTCTCA
F-L-Y-S-K-L-T-V-D-K-S-R-N-Q-Q-G-N-V-P-S-
TGCTCCGTGATGATGAGGCTCTGCACAACTACACGCAAGAGGCTCTCCCTGTCT
C-S-V-H-H-E-A-L-H-N-H-Y-T-Q-X-S-L-S-L-S-
CCGGGTAAATGA
P-Q-K-

Figure 22

Amix -26
Nucleotide -78

(M) TGGGTG TACTGCTCACACAGGACGCTGCTCAGTCTGGTCCCTGCACTCCTGTTTCCA
G V L L T Q R T L L S L V L A L L F P
+1
AGCATGCCGAGCATGGCAATGCACGTGCCCCAGCCTGCTGTGGTACTGCCAGCAGCCGA
S M A S M A M H V A Q P A V V L A S S R
GGCATCGCTAGCTTTGTGTGTGAGTATGCATCTCCAAGCAAAGCCACTGAGGTCCGGGTG
G I A S P V C E Y A S P G K A T E V R V
ACAGTGCTTCGGCAGGCTGACAGCCAGGTGACTGAAGTCTGTGGGCAACCTACATGATG
T V L R Q A D S Q V T E V C A A T Y N M
GGGAATGAGTTGACCTTCCTAGATGATTCCATCTGCACGGGCACCTCCAGTGAAATCAA
G N E L T F L D D S I C T G T S S G N Q
GTGAACCTCACTATCCAAGGACTGAGGCCCATGGACACGGGACTCTACATCTGCAAGGTG
V N L T I Q G L R A M D T G L Y I C K V
GAGCTCATGTACCCACCGCCATACCTAGAGGGCATAGGCAACGGAAACCCAGATTATGTA
E L M Y P P P Y Y E G I G N G T Q I Y V
ATTGATCCAGAACCGTGCCCAAGATTCTGATCAAGAGCCCAAATCTTCTGACAAAACCTCAC
I D P E P C P D S D Q E P K S S D K T H
ACATCCCCACCGTCCCCAGCACCTGAACTCCTGGGGGGATCGTCACTCTTCTCTTCCCC
T S P P S P A P E L L G G S S V F L P P
CCAAAACCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTACATCCGTGCTGGT
P K P K D T L M I S R T P E V T C V V V
GACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGCTACGTGACCGCGGTGGAGGTG
D V S H E D P E V K F N W Y V D G V E V
CATAATGCCAAGACAAAGCCGGGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGC
H N A K T K P R E E Q Y N S T Y R V V S
GTCTCACCGTCTGCAACGAGTGGCTGAATGGCAAGGAGTACAAGTCAAGGTCTCTCC
V L T V L H Q D W L N G K E Y K C K V S
AACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGOCAGCCCCGA
N K A L P A P I E K T I S K A K G Q P R
GAACCACAGGTGTACACCCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAAC
E P Q V Y T L P P S R D E L T K N Q V S
CTGACCTGCTGTGCAAAAGCTTCTATCCCAAGGACATCGCCGTGGAGTGGGAGAGCAAT
L T C L V K G F Y P S D I A V E W E S N
GGGCAGCCGGAGAACAACTACAAGACCACGCTCCCGTCTGGACTCCGACGGCTCCTTC
G Q P E N N Y K T T P P V L D S D G S F
TTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCA
F L Y S K L T V D K S R W Q Q G N V F S
TGCTCCGTGATGCATGAAGCTCTGCACAACCACTACACGACAGAAGACCTCTCCCTGTCT
C S V M H E A L H N N Y T Q K S L S L S
CCGGGTAAATGA
P G K

Figure 23

amino acid
-26
nucleotide
-78



ATGGGTGTACTGCTCACACAGAGGACGCTGCTCAGTCTGGTCCTTGCCTCTCTTCCA
(H) G V L L T Q R T L L S L V L A L L 7 P
AGCATGGCGAGCATGGCAATGCACGTGGCCAGCCTGCTGTGTACTGGCCAGCAGCGA
S H A S M A H V A Q P A V V L A S S R
NRCL XL +1
GGCATCGCTAGCTTTGTGTGTGAGTATGCATCTCCAGGCAAATATCTAGAGTCCGGGTG
G I A S P V C E Y A S P G K Y T E V R V
ACAGTGTCTCGGCAGGCTGACAGCCAGGTGACTGAAGTCTGTGCGGCAACCTACATGATG
T V L R Q A D S Q V T E V C A A T Y M M
GGGAATGAGTTGACCTTCTAGATGATTCCATCTGCACGGGCACCTCCAGTGGAATCAA
G N E L T P L D D S I C T G T S S G N Q
GTGAACCTCACTATCCAAGGACTGAGGGCCATGGACACGGGACTCTACATCTGCAAGGTG
V N L T I Q G L R A M D T G L Y I C K V
GAGCTCATGTACCCACCGCCATACTACGAGGGCATAGGCAACGGAACCCAGATTATGTA
E L M Y P P P Y Y E G I G N G T O I Y V
ATTGATCCAGAACCOTGCCAGATTCTGATCAGGAGCCCAAATCTCTGACAAACCTCAG
I D P E P C P D S D Q E P K S S D K T H
ACATCCCCACCGTCCCCAGCACCTGAACCTCTGGGGGATCGTCACTCTTCTCTTCCCC
T S P P S P A P E L L G G S S V P L F P
CCAAACCCCAAGGACACCCTCATGATCTCCCGGACCCCTGAAGTCAATGCCGTGGTGGTG
P K P K D T L M I S R T P E V T C V V V
GACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGCGCTGGAGGTG
D V S H E D P E V K F N W Y V D G V E V
CATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGC
H N A K T K P R E E Q Y N S T Y R V V S
GTCCTACCGTCTCGCACCAGGACTGGCTGAATGGCAAGGAGTACAAOTGCAAGGTCTCC
V L T V L H Q D W L N G K E Y K C X V S
AACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGCGAGCCCCGA
N K A L P A P I E K T I S K A K G Q P R
GAACCACAGGTGTACACCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTACGC
E P Q V Y T L P P S R D E L T K N Q V S
CTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT
L T C L V K G F Y P S D I A V E W E S N
GGGCAGCCCGAGAACAACTACAAGACCACGCCCTCCCGTGTGGACTCCGACGCTCTCTC
G Q P E N N Y K T T P P V L D S D G S P
TTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGAACGTCTCTCA
P L Y S K L T V D K S R W Q Q G N V F S
TGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAAGAGGCTCTCCCTGTCT
C S V M H E A L H N H Y T Q K S L S L S
CCGGTAAATGA
P G K

L104EA29L I₃

1

ATGGGTGTAAGTGTACACAGAGGACGCTGCTCAGTCTGGTCCCTTGCACTCCTGTTTCCA
M--G--V--L--L--T--Q--R--T--L--L--S--L--V--L--A--L--L--F--P--
AGCATGGCGAGCATGGCAATGCACGTGGCCAGCCTGCTGTGGTACTGGCCAGCAGCCGA
S--M--A--S--M--A--M--H--V--A--Q--P--A--V--V--L--A--S--S--R--
GGCATCGCTAGCTTTGTGTGTGATGATGTCATCTCCAGGCAAATTGACTGAGGTCCGGGTG
G--I--A--S--F--V--C--E--Y--A--S--P--G--K--L--T--E--V--R--V--
ACAGTGCTTCGGCAGGCTGACAGCCAGGTGACTGAAGTCTGTGCGCAACCTACATGATG
T--V--L--R--Q--A--D--S--Q--V--T--E--V--C--A--A--T--Y--M--M--
GGGAATGAGTTGACCTTCCTAGATGATTCCATCTGCACGGGCACCTCCAGTGGAATCAA
G--N--B--L--T--F--L--D--D--S--I--C--T--G--T--S--S--G--N--Q--
GTGAACCTCACTATCCAAGGACTGAGGGCCATGGACACGGGACTCTACATCTGCAAGGTG
V--N--L--T--I--Q--G--L--R--A--M--D--T--G--L--Y--I--C--K--V--
GAGCTCATGTACCCACCGCCATACTACGAGGGCATAGGCAACGGAACCCAGATTTATGTA
E--L--M--Y--P--P--P--Y--Y--E--G--I--G--N--G--T--Q--I--Y--V--
ATTGATCCAGAACCGTGCCAGATTCTGATCAGGAGCCCAAATCTCTGACAAAACCTCAC
I--D--P--E--P--C--P--D--S--D--Q--E--P--K--S--S--D--K--T--H--
ACATCCCCACCGTCCCCAGCACCTGAACTCCTGGGGGATCGTCAGTCTTCTCTTCCCC
T--S--P--P--S--P--A--P--E--L--L--G--G--S--S--V--F--L--F--P--
CCAAAACCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTG
P--K--P--K--D--T--L--M--I--S--R--T--P--E--V--T--C--V--V--V--
GACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTG
D--V--S--H--E--D--P--E--V--K--F--N--W--Y--V--D--G--V--E--V--
CATAATGCCAAGACAAAGCCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGC
H--N--A--K--T--K--P--R--E--E--Q--Y--N--S--T--Y--R--V--V--S--
GTCCTCACCGTCTCGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC
V--L--T--V--L--H--Q--D--W--L--N--G--K--E--Y--K--C--K--V--S--
AACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA
N--K--A--L--P--A--P--I--E--K--T--I--S--K--A--K--G--Q--P--R--
GAACCACAGGTGTACACCCCTGCCCCCATCCCGGATGAGCTGACCAAGAACCAGGTCAGC
E--P--Q--V--Y--T--L--P--P--S--R--D--E--L--T--K--N--Q--V--S--
CTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT
L--T--C--L--V--K--G--F--Y--P--S--D--I--A--V--E--W--E--S--N--
GGGCAGCCGGAGAACAACCTACAAGACCACGCTCCCGTGTGGACTCCGACGGCTCCTTC
G--Q--P--E--N--N--Y--K--T--T--P--P--V--L--D--S--D--G--S--F--
TTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCA
F--L--Y--S--K--L--T--V--D--K--S--R--W--Q--Q--G--N--V--F--S--
TGCTCCGTGATGATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCT
C--S--V--M--H--E--A--L--H--N--H--Y--T--Q--K--S--L--S--L--S--
CCGGGTAAATGA
P--G--K--

Figure 25



125-277-11

Figure 26

L104EA29WJg

ATGGGTGTACTGCTCACACAGAGGACGCTGCTCAGTCTGGTCTGCACTCTCTTTCCA
M--G--V--L--L--T--Q--R--T--L--L--S--L--V--L--A--L--L--P--P--
AGCATGGCGAGCATGGCAATGCACGTGGCCAGCCTGCTGTGGTACTGGCCAGCAGCCGA
S--M--A--S--M--A--M--H--V--A--Q--P--A--V--V--L--A--S--S--R--
GGCATCGCTAGCTTTGTGTGTGAGTATGCATCTCCAGGCAAATGGACTGAGGTCCGGGTG
G--I--A--S--F--V--C--E--Y--A--S--P--G--K--W--T--E--V--R--V--
ACAGTGCCTTCGGCAGGCTGACAGCCAGGTGACTGAAGTCTGTGCGGCAACCTACATGAT
T--V--L--R--Q--A--D--S--Q--V--T--E--V--C--A--A--T--Y--M--M--
GGGAATGAGTTGACCTTCCTAGATGATTCCATCTGCACGGGCACCTCCAGTGGAAATCAA
Q--N--B--L--T--F--L--D--D--S--I--C--T--G--T--S--S--G--N--Q--
GTGAACCTCACTATCCAAGGACTGAGGGCCATGGACACGGGACTCTACATCTGCAAGGTG
V--N--L--T--I--Q--G--L--R--A--M--D--T--G--L--Y--I--C--K--V--
GAGCTCATGTACCCACCGCCATACTACGAGGGCATAGGCAACGGAACCCAGATTATGTA
E--L--M--Y--P--P--P--Y--Y--E--G--I--G--N--G--T--Q--I--Y--V--
ATTGATCCAGAACCGTGCCAGATTCTGATCAAGAGCCCAAATCTTCTGACAAAACTCAC
I--D--P--B--P--C--P--D--S--D--Q--B--P--K--S--S--D--K--T--H--
ACATCCCCACCGTCCCCAGCACCTGAACCTCTGGGGGGATCGTCAGTCTTCTCTTCCCC
T--S--P--P--S--P--A--P--E--L--L--G--G--B--S--V--F--L--P--P--
CCAAAACCCCAAGGACACCTCATGATCTCCCGACCCCTGAGGTCACATGCGTGGTGGTG
P--K--P--K--D--T--L--M--I--S--R--T--P--E--V--T--C--V--V--V--
GACGTGAGCCACGAAGACCCCTGAGGTCAGTCAAGTCAACTGGTACGTGGACGGCGTGGAGGTG
D--V--S--H--E--D--P--E--V--K--F--N--W--Y--V--D--G--V--B--V--
CATAATGCCAAGACAAAGCCCGGGGAGGAGCAOTACAACAGCAGCTACCGTGTGGTCAGC
H--N--A--X--T--K--P--R--E--E--Q--Y--N--S--T--Y--R--V--V--S--
GTCTCACCCTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC
V--L--T--V--L--H--Q--D--W--L--N--G--K--E--Y--K--C--K--V--S--
AACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGCAGCCCCGA
N--K--A--L--P--A--P--I--E--K--T--I--S--K--A--K--G--Q--P--R--
GAACCACAGGTGTACACCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTGAGC
E--P--Q--V--Y--T--L--P--P--S--R--D--E--L--T--K--N--Q--V--S--
CTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT
L--T--C--L--V--K--G--F--Y--P--S--D--I--A--V--E--W--E--S--N--
GGGCAGCCGGGAGAACTACAAGACCACGCCTCCCGTGGTGGACTCCGACGGCTCTCTC
G--Q--P--E--N--N--Y--K--T--T--P--P--V--L--D--S--D--G--S--F--
TTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTCTCA
F--L--Y--S--K--L--T--V--D--K--S--R--W--Q--Q--G--N--V--P--S--
TGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCT
C--S--V--M--H--E--A--L--H--N--H--Y--T--Q--K--S--L--S--L--S--
CCGGGTAAATGA-----
P--G--K-----

Figure 27

Equilibrium binding to CD86Ig

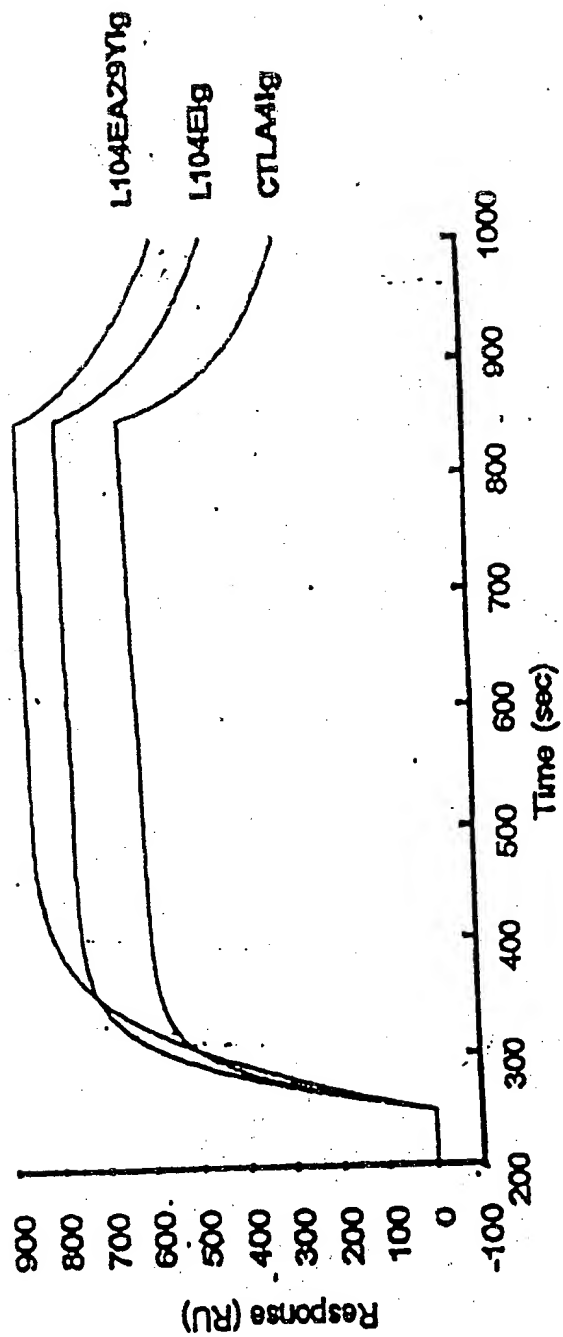


Figure 28

Fig. 29A

Human CD80 CHO cells

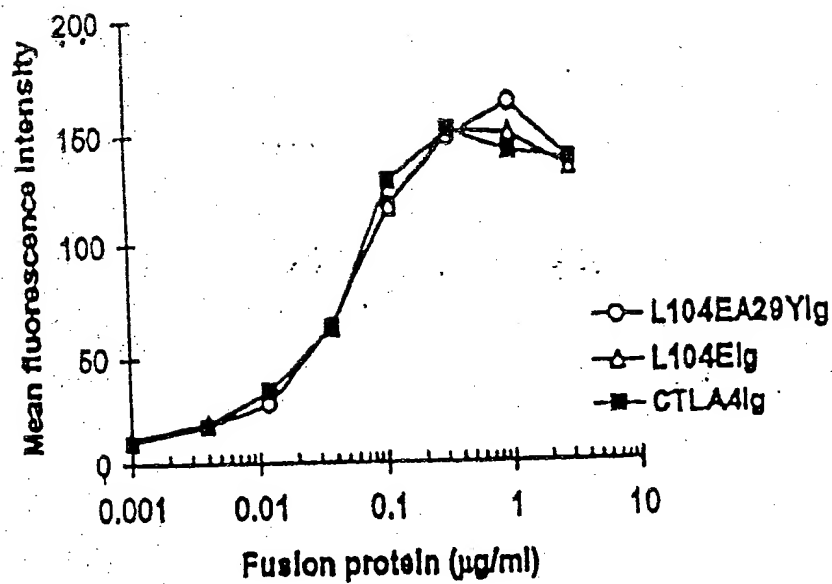


Fig. 29B

Human CD86 CHO cells

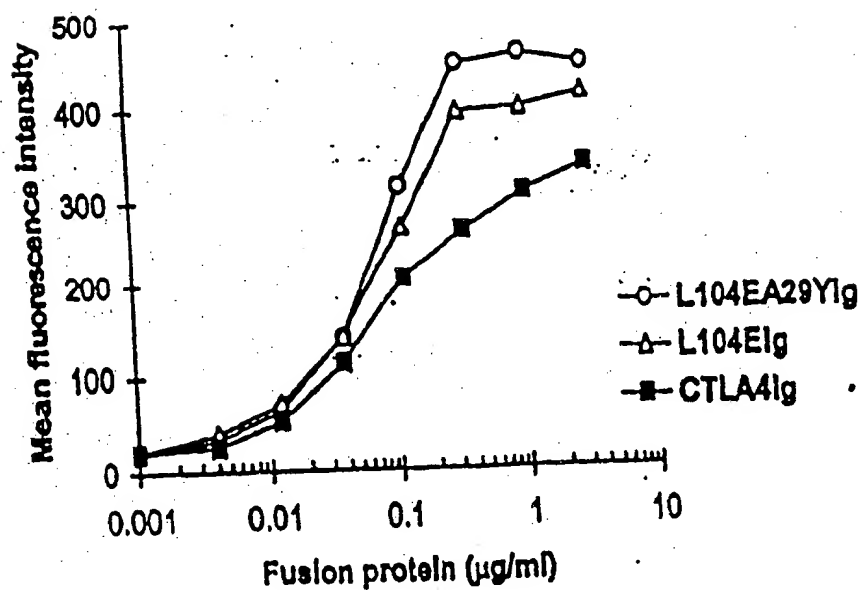


Fig. 30A CD80 CHO + PMA costimulation

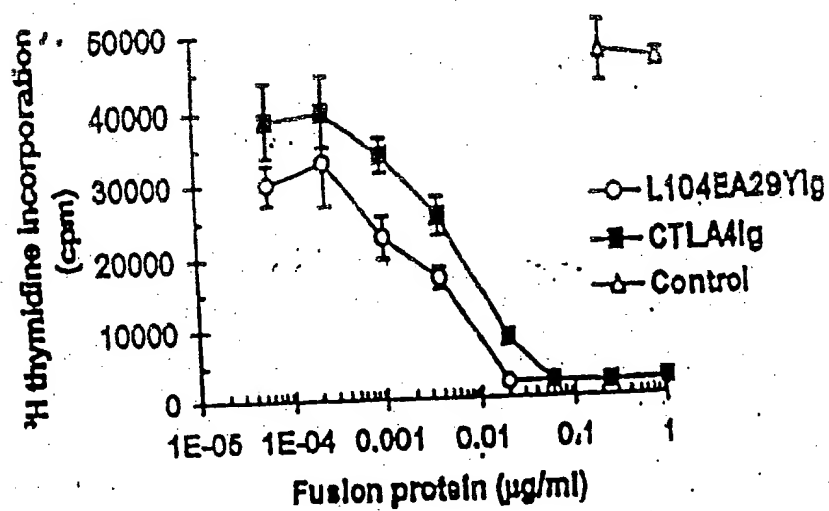


Fig. 30B CD86 CHO + PMA costimulation

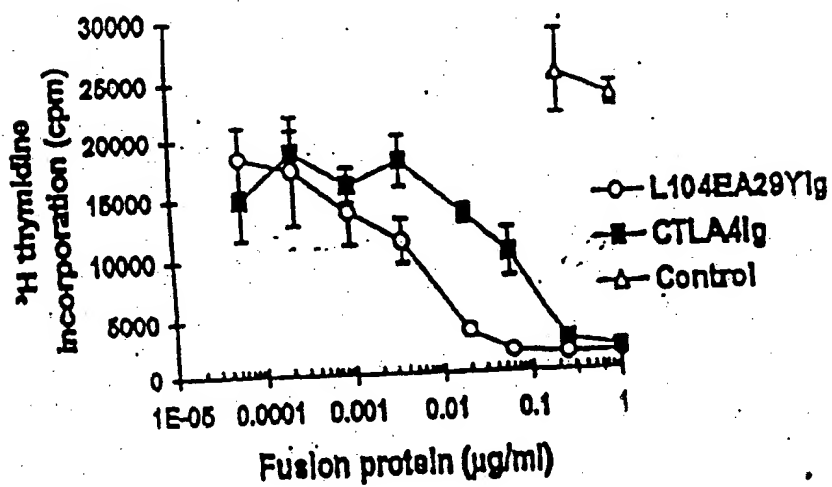


Fig. 3/ A Primary alloresponse

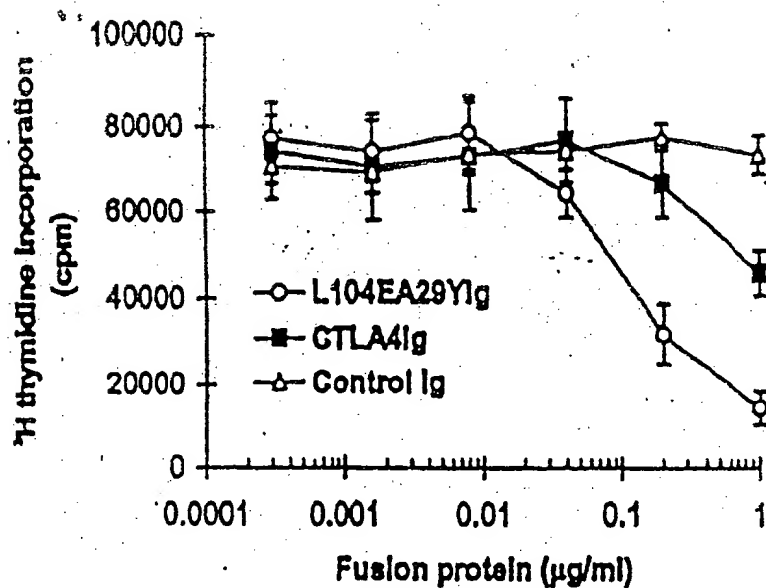


Fig. 3/ B Secondary alloresponse

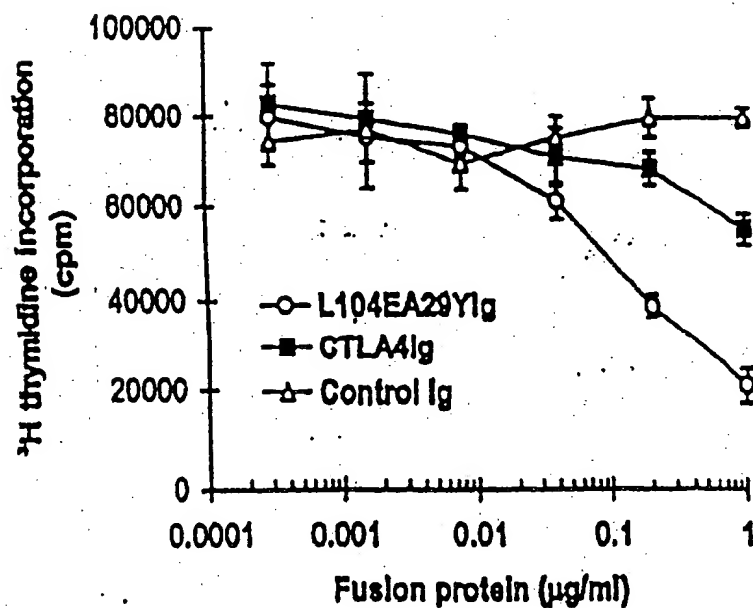


Fig. 32 A

IL-2

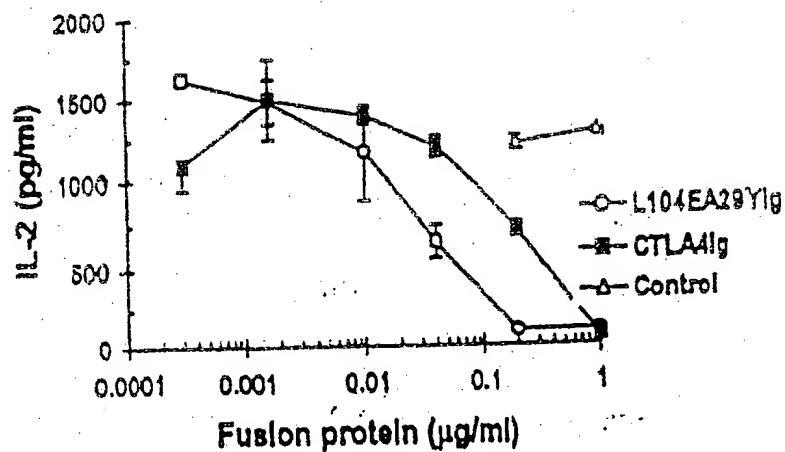


Fig. 32 B

IL-4

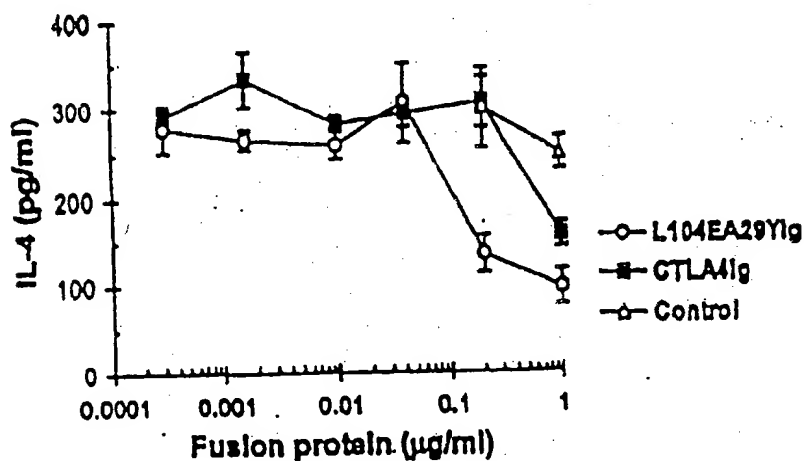


Fig. 32 C

γ-IFN

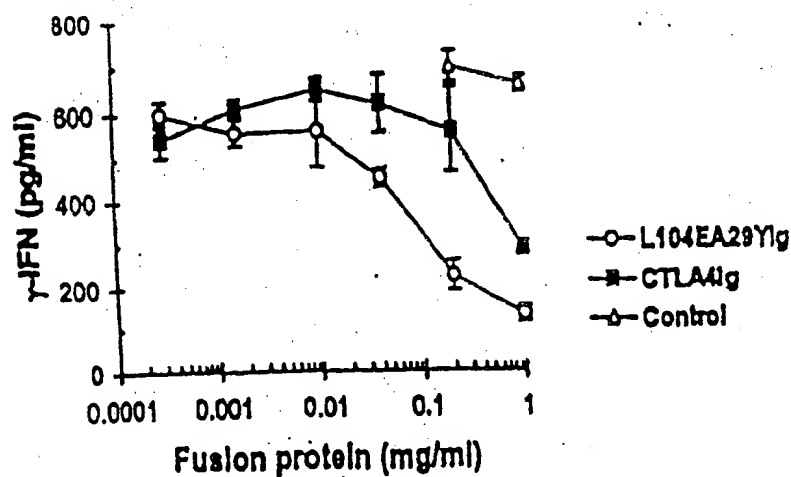


Fig. 33 Inhibition of PHA-Induced monkey T cell proliferation

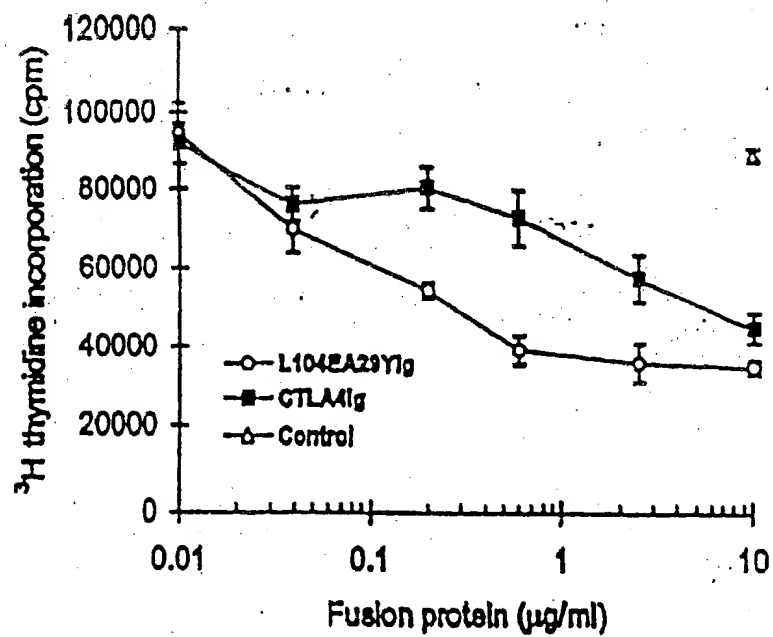


Fig 34A

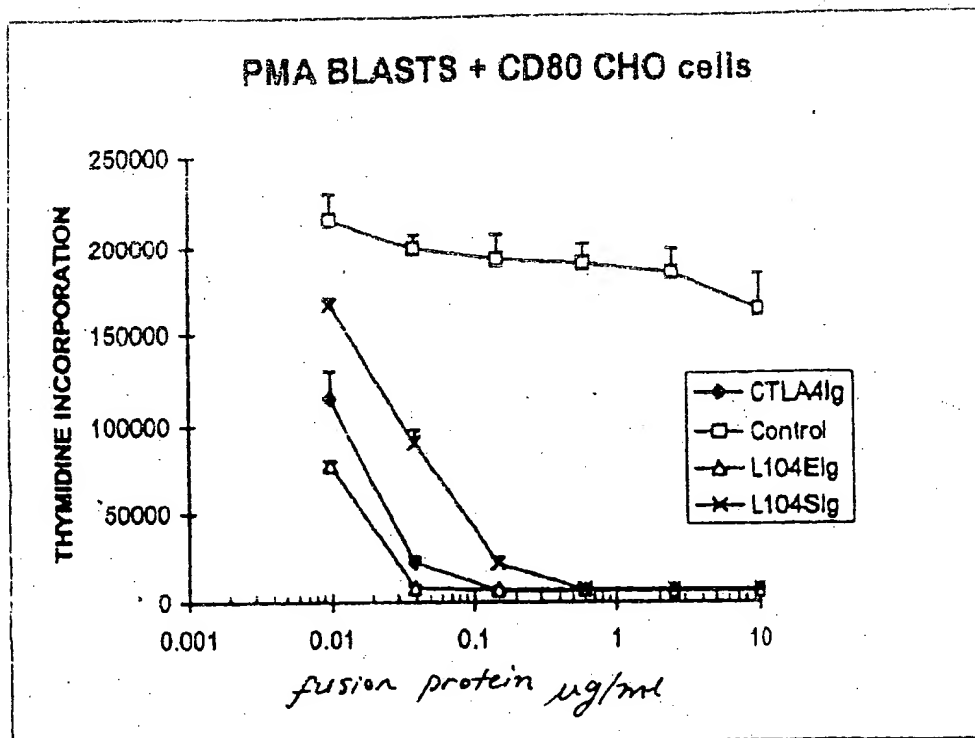


Fig. 34B

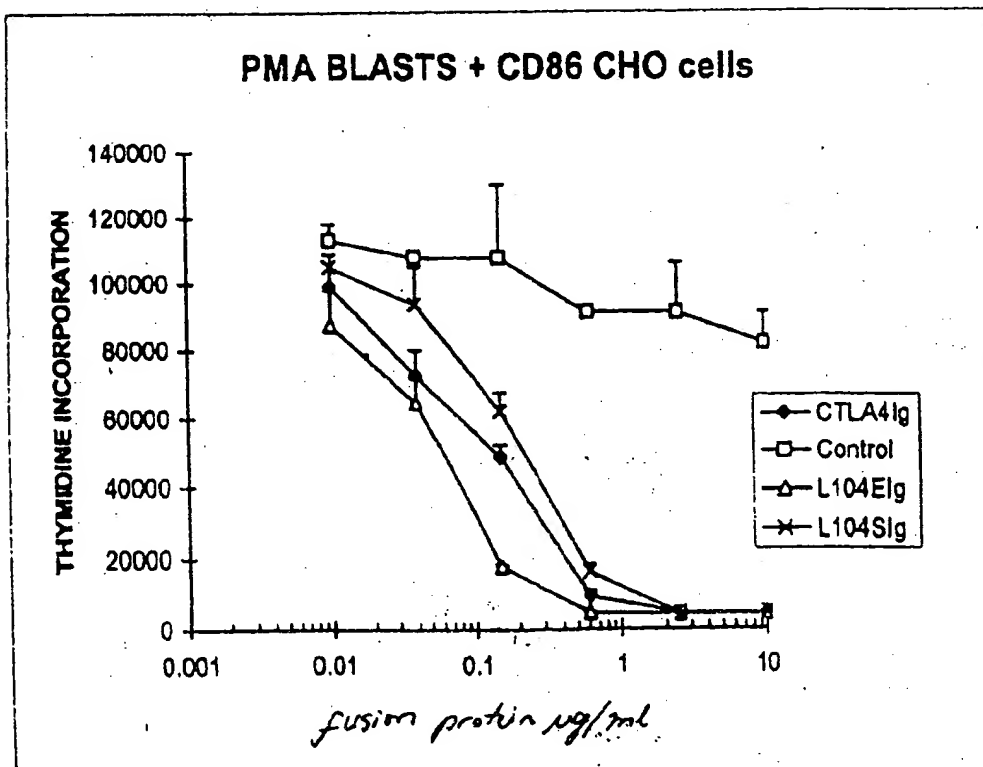


Fig. 35A

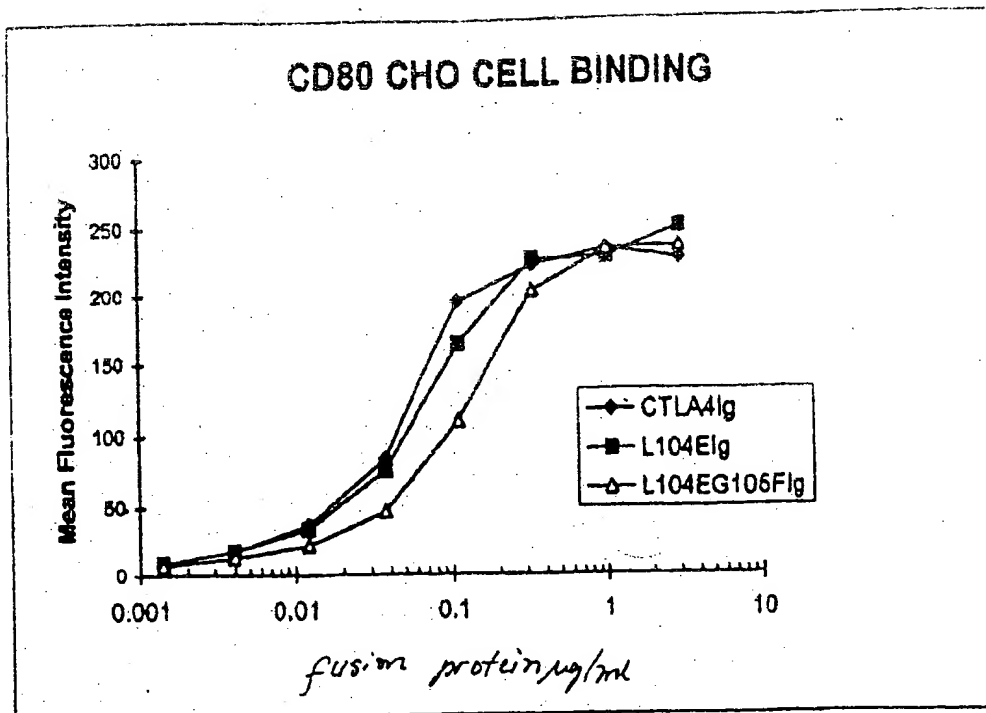


Fig. 35B

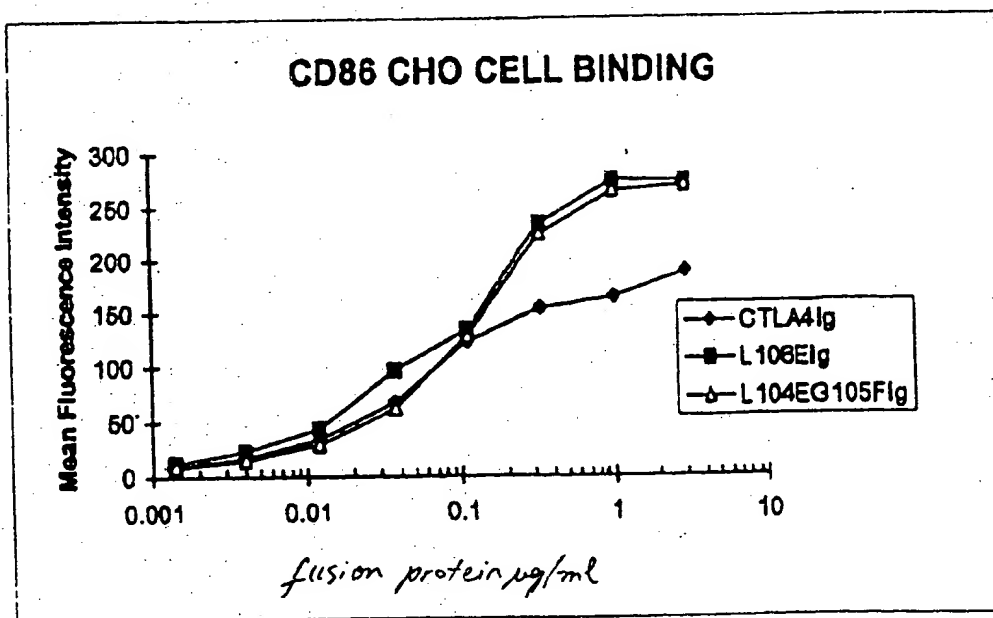


Fig 36

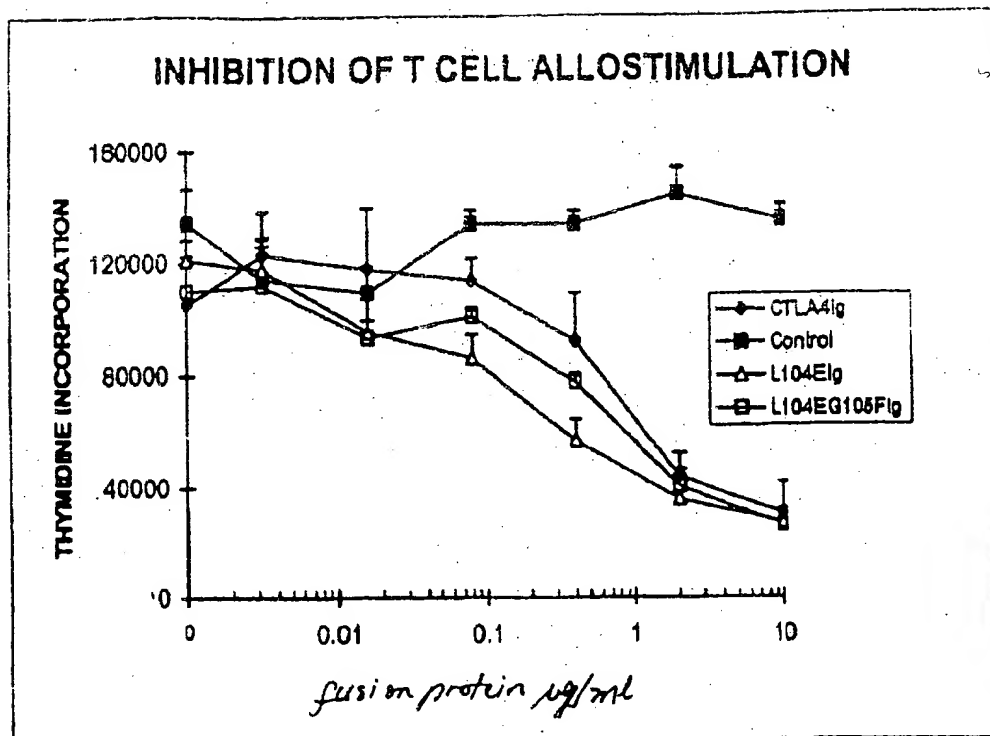


Fig. 37 A

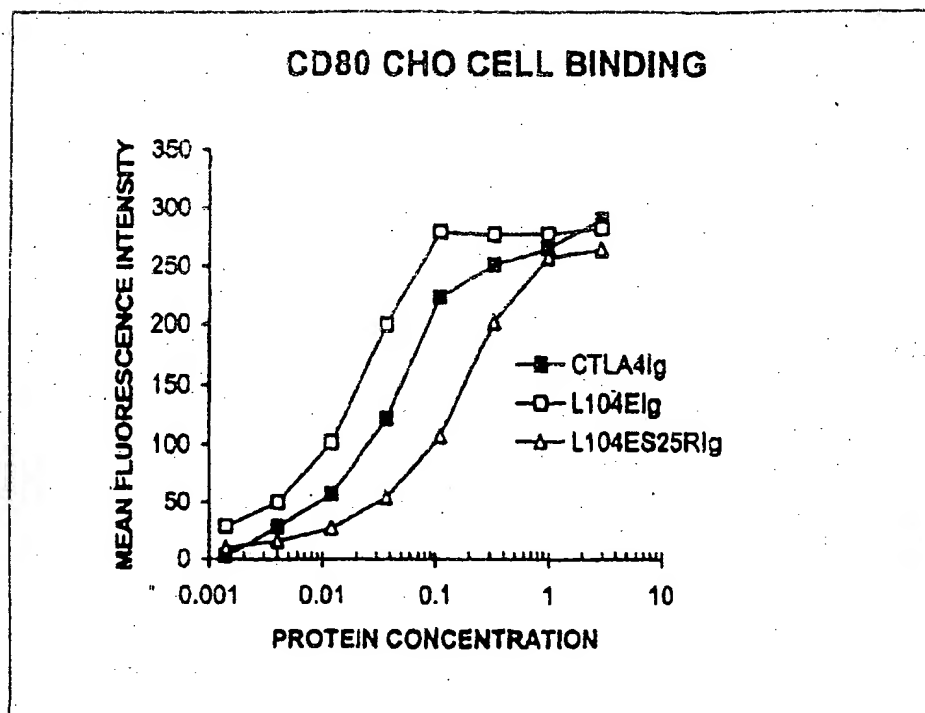
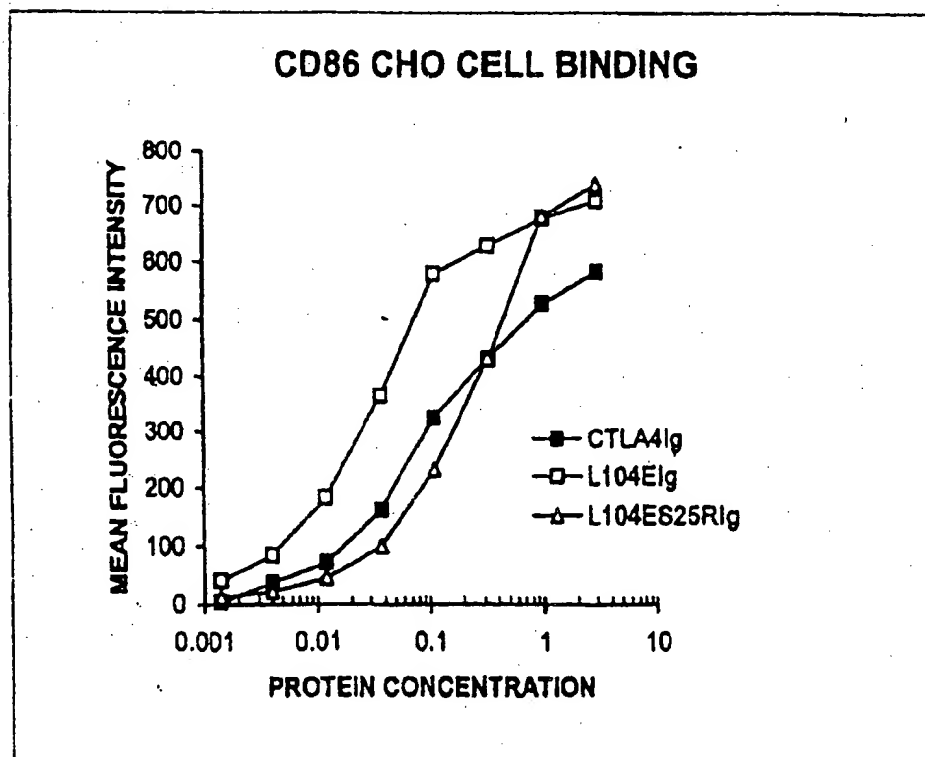


Fig. 37 B



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley, et al.
Serial No. : 09/609,915 Examiner: E. M. Lazar-Wesley, Ph.D.
Filed : July 3, 2000 Group Art Unit: 1646
For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES
THEREOF

35 North Arroyo Pkwy, Suite 60
Pasadena, California 91103
April 15, 2002

Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

PRELIMINARY AMENDMENT

Please amend the subject application as follows.

In the Specification:

In accordance with 37 C.F.R. 1.121(a)(1)(i) and (ii), please replace the paragraph at page 1, lines 5-10, with the following rewritten paragraph:

-- This application is a continuation-in-part of U.S. Serial No. 08/228,208, filed April 15, 1994, now U.S. Patent No. 6,090,914, issued July 18, 2000, which was a continuation-in-part of U.S. Serial No. 08/008, 898, filed January 22, 1993, now U.S. Patent No. 5,770,197, issued June 23, 1998, which was a continuation-in part of U.S. Serial No. 07/723,617, filed June 27, 1991, now abandoned; U.S. Serial No. 09/603,825, filed June 26, 2000, which was a continuation-in-part of U.S. Serial No. 09/014,761, filed January 28, 1998, which claims priority of U.S. Serial No. 60/036,594, filed January 31, 1997, now abandoned; and U.S. Serial No. 08/539,436, filed October 5, 1995, now U.S. Patent No. 6,132,992, issued

October 17, 2000, the contents of all of which are hereby incorporated by reference, in their entirety, into the present application. --

Please replace the paragraph at page 4, lines 14-16, beginning "Figure 3:", with the following rewritten paragraph:

-- Figure 3: Shows the nucleotide (SEQ ID NO.: 1) and complete amino acid sequence (SEQ ID NO.: 2) encoding human CTLA4 receptor fused to the oncostatin M signal peptide, and including the newly identified N-linked glycosylation site, as described in Example 1, infra. --

Please replace the paragraph at page 6, lines 20-25, please replace the paragraph beginning "Figure 7:", with the following rewritten paragraph:

-- Figure 17: Depicts sequencing alignment of CD28 and CTLA4 family members. Sequences of human (H) (SEQ ID NO.: 7), mouse (M) (SEQ ID NO.: 5), rat (R) (SEQ ID NO.: 6), and chicken (Ch) CD28 (SEQ ID NO.: 8) are aligned with human (SEQ ID NO.: 3) and mouse CTLA4 (SEQ ID NO.: 4). The signal peptides are underlined with a dashed line. The transmembrane domains are underlined with a solid line. The CDR-analogous regions are noted. The dark shaded areas highlight complete conservation of residues while the light shaded areas highlight conservative amino acid substitutions in all family members.--

Please replace the paragraph at page 7, lines 12-13, beginning "Figure 22:", with the following rewritten paragraph:

-- Figure 22: Depicts the nucleotide (SEQ ID NO.: 9) and amino acid sequence of a CTLA4Ig (SEQ ID NO.: 10) having wildtype extracellular domain of CTLA4. --

Please replace the paragraph at page 7, lines 15-17, beginning "Figure 23:", with the following rewritten paragraph:

-- Figure 23: Depicts the nucleotide (SEQ ID NO.: 11) and amino acid (SEQ ID NO.: 12) sequences of L104EIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.--

Please replace the paragraph at page 7, lines 19-21, beginning "Figure 24:", with the following rewritten paragraph:

-- Figure 24: Depicts the nucleotide (SEQ ID NO.: 13) and amino acid sequence (SEQ ID NO.: 14) of L104EA29YIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.--

Please replace the paragraph at page 7, lines 23-25, beginning "Figure 25:", with the following rewritten paragraph:

-- Figure 25: Depicts the nucleotide (SEQ ID NO.: 15) and amino acid sequences (SEQ ID NO.: 16) of L104EA29LIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.--

Please replace the paragraph at page 7, lines 27-29, beginning "Figure 26:", with the following rewritten paragraph:

-- Figure 26: Depicts the nucleotide (SEQ ID NO.: 17) and amino acid sequences (SEQ ID NO.: 18) of L104EA29TIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.--

Please replace the paragraph at page 8, lines 1-3, beginning "Figure 27:", with the following rewritten paragraph:

-- Figure 27: Depicts the nucleotide (SEQ ID NO.: 19) and amino acid sequences (SEQ ID NO.: 20) of L104EA29WIIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.--

Please replace the paragraph at page 9, lines 22-25, beginning "Figure 37:", with the following rewritten paragraph:

-- Figure 37: Depicts the results of a FACS assay, showing L104EIIg and L104ES25RIg bind CHO cells stably transfected with human CD80 or CD86. A) L104EIIg and

L104ES25RIg bind to human CD80 CHO-transfected cells; B) L104EIg and L104ES25RIg bind to human CD86 CHO-transfected cells.--

Please replace the paragraph at page 11 lines 1-8, beginning "One embodiment", with the following rewritten paragraph:

-- One embodiment of a soluble CTLA4 has been deposited with the American Type Culture Collection (ATCC) in Manassas, Maryland, under the provisions of the Budapest Treaty on May 31, 1991 and has been accorded ATCC accession number: 68629. ATCC 68629 is DNA encoding *CTLA4* Ig. Additionally, the CTLA4Ig-24 CHO cell line has been deposited with the ATCC under the Budapest Treaty on May 31, 1991 and has been accorded accession number ATCC 10762. DNA encoding L104EA29YIg was submitted for deposit on June 19, 2000 with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209. The DNA encoding L104EA29YIg has been accorded ATCC accession number PTA-2104. --

Please replace the paragraph at page 33, lines 2-21, beginning "Because a signal peptide", with the following rewritten paragraph:

-- Because a signal peptide for CTLA4 was not identified in the CTLA4 gene, the N-terminus of the predicted sequence of CTLA4 was fused to the signal peptide of oncostatin M (Malik et al., Mol. and Cell. Biol. 9:2847 (1989)) in two steps using overlapping oligonucleotides.

For the first step, the oligonucleotide,
CTCAGTCTGGTCCTTGCACTCCTG

TTTCCAAGCATGGCGAGCATGGCAATGCACGTGGCCCAGCC (SEQ ID NO.: 21)

(which encoded the C terminal 15 amino acids from the oncostatin M signal peptide fused to

the N terminal 7 amino acids of CTLA4) was used as forward primer, and

TTTGGGCTCCTGATCAGAATCTGGGCACGGTTG (SEQ ID NO.: 22) (encoding

amino acid residues 119-125 of the amino acid sequence encoding CTLA4 receptor and

containing a Bcl I restriction enzyme site) as reverse primer. The template for this step was

cDNA synthesized from 1 micro g of total RNA from H38 cells (an HTLV II infected T cell

leukemic cell line provided by Drs. Salahudin and Gallo, NCI, Bethesda, MD). A portion of

the PCR product from the first step was reamplified, using an overlapping forward primer, encoding the N terminal portion of the oncostatin M signal peptide and containing a Hind III restriction site, endonuclease

CTAGCCACTGAAGCTTCACCAATGGGTGTACTGCTCACACAGAGGACGCTGCT
CAGTCTGGTCCTTGCACTC (SEQ ID NO.: 23) and the same reverse primer. The product of the PCR reaction was digested with Hind III and Bcl I and ligated together with a Bcl I/Xba I cleaved cDNA fragment encoding the amino acid sequences corresponding to the hinge, CH2 and CH3 regions of IgC γ 1 into the Hind III/Xba I cleaved expression vector, CDM8 or Hind III/Xba I cleaved expression vector pLN. --.

Please replace the paragraph at page 33, lines 23-29, beginning "A schematic map", with the following rewritten paragraph:

-- A schematic map of the resulting CTLA4Ig fusion construct is shown in Figure 1. Sequences displayed in this figure show the junctions between CTLA4 (upper case letters, unshaded regions) and the signal peptide, SP, of oncostatin M (dark shaded regions), and the hinge, H, of IgC(gamma)1 (stippled regions). The amino acid in parentheses was introduced during construction. Asterisks (*) indicate cysteine to serine mutations introduced in the IgC γ hinge region. The immunoglobulin superfamily V-like domain present in CTLA4 is indicated, as are the CH2 and CH3 domains of IgC(gamma)1. --.

Please replace the paragraph at page 34, lines 21-27, beginning "CTLA4Ig", with the following rewritten paragraph:

-- CTLA4Ig was purified by protein A chromatography from serum-free conditioned supernatants (Figure 2). Concentrations of CTLA4Ig were determined assuming an extinction coefficient at 280 nm of 1.6 (experimentally determined by amino acid analysis of a solution of known absorbance). Molecular weight standards (lanes 1 and 3, Figure 2) and samples (1 micro grams) of CTLA4Ig (lanes 2 and 4) were subjected to SDS-PAGE (4-12% acrylamide gradient) under non-reducing conditions (- beta ME, lanes 1 and 2) or

reducing conditions (+ beta ME, lanes 3 and 4) Proteins were visualized by staining with Coomassie Brilliant Blue. --.

Please replace the paragraph at page 35, lines 19-31, beginning "Because of expression of CTLA4 receptor", with the following rewritten paragraph:

-- Because the expression of CTLA4 receptor protein in human lymphoid cells has not been previously reported, it was necessary to locate a source of CTLA4 mRNA. cDNA was reverse transcribed from the total cellular RNA of H38 cells, as noted above, was used for cloning by PCR. For this purpose, the oligonucleotide, GCAATGCACGTGGCCCAGCCTGCTGTGGTAGTG (SEQ ID NO.: 24) (encoding the first 11 amino acids in the predicted coding sequence) was used as a forward primer, and TGATGTAACATGTCTAGATCAATTGATGGGAATAAAATAAGGCTG (SEQ ID NO.: 25) (homologous to the last 8 amino acids in CTLA4 and containing a Xba I site) as reverse primer. The template again was a cDNA synthesized from 1 micro gram RNA from H38 cells. Products of the PCR reaction were cleaved with the restriction endonucleases Nco I and Xba I and the resulting 316 bp product was gel purified. A 340 bp Hind III/Nco I fragment from the CTLA4Ig fusion described above was also gel-purified, and both restriction fragments were ligated into Hind III/Xba I cleaved CDM8 to form OMCTLA. --.

Please replace the paragraph at page 36, lines 21-25, beginning "Receptor-immunoglobulin C gamma", with the following rewritten paragraph:

-- Receptor-immunoglobulin C gamma (IgC γ) fusion proteins B7Ig and CD28Ig were prepared as described by Linsley et al., in J. Exp. Med. 173:721-730 (1991), incorporated by reference herein. Briefly, DNA encoding amino acid sequences corresponding to the respective receptor protein (e.g. B7) was joined to DNA encoding amino acid sequences corresponding to the hinge, CH2 and CH3 regions of human IgC γ 1. This was accomplished as follows. --.

Please replace the paragraph at page 37, lines 6-25, beginning "Plasmid Construction," with the following rewritten paragraph:

-- Plasmid Construction. Expression plasmids containing cDNA encoding CD28, (as described by Aruffo and Seed, Proc. Natl. Acad. Sci. USA 84:8573 (1987)), were provided by Drs. Aruffo and Seed (Mass General Hospital, Boston, MA). Plasmids containing cDNA encoding CD5, (as described by Aruffo, Cell 61:1303 (1990)), were provided by Dr. Aruffo. Plasmids containing cDNA encoding B7, (as described by Freeman et al., J. Immunol. 143:2714 (1989)), were provided by Dr. Freeman (Dana Farber Cancer Institute, Boston, MA). For initial attempts at expression of soluble forms of CD28 and B7, constructs were made (OMCD28 and OMB7) as described by Linsley et al., J. Exp. Med., *supra*, in which stop codons were introduced upstream of the transmembrane domains and the native signal peptides were replaced with the signal peptide from oncostatin M (Malik et al., Mol. Cell Biol. 9:2847 (1989)). These were made using synthetic oligonucleotides for reconstruction (OMCD28) or as primers (OMB7) for PCR. OMCD28, is a CD28 cDNA modified for more efficient expression by replacing the signal peptide with the analogous region from oncostatin M. CD28Ig and B7Ig fusion constructs were made in two parts. The 5' portions were made using OMCD28 and OMB7 as templates and the oligonucleotide, CTAGCCACTGAAGCTTCACCATGGGTGTACTGCTCACAC (SEQ ID NO.: 26) (encoding the amino acid sequence corresponding to the oncostatin M signal peptide) as a forward primer, and either TGGCATGGGCTCCTGATCAGGCTTAGAAGGTCCGGGAAA (SEQ ID NO.: 27) or, TTTGGGCTCCTGATCAGGAAAATGCTCTTGCTTGGTTGT (SEQ ID NO.: 28) as reverse primers, respectively. Products of the PCR reactions were cleaved with restriction endonucleases (Hind III and BclI) as sites introduced in the PCR primers and gel purified.--

Please replace the paragraph at page 37, line 27, through page 38, line 9, beginning "The 3' portion of the fusion constructs", with the following rewritten paragraph:

-- The 3' portion of the fusion constructs corresponding to human IgC γ 1 sequences was made by a coupled reverse transcriptase (from Avian myeloblastosis virus; Life Sciences

Associates, Bayport, NY)-PCR reaction using RNA from a myeloma cell line producing human-mouse chimeric mAb L6 (provided by Dr. P. Fell and M. Gayle, Bristol-Myers Squibb Company, Pharmaceutical Research Institute, Seattle, WA) as template. The oligonucleotide,

AAGCAAGAGCATTTTCCTGATCAGGAGCCCCAAATCTTCTGACAAAACCTCACA
CATCCCCACCGTCCCCAGCACCTGAACTCCTG (SEQ ID NO.: 29) was used as
forward primer,

CTTCGACCAGTCTAGAAGCATCCTCGTGCGACCGCGAGAGC (SEQ ID NO.: 30)
as reverse primer. Reaction products were cleaved with BclI and XbaI and gel purified. Final constructs were assembled by ligating HindIII/BclI cleaved fragments containing CD28 or B7 sequences together with BclI/XbaI cleaved fragment containing IgCy1 sequences into HindIII/XbaI cleaved CDM8. Ligation products were transformed into MC1061/p3 E. coli cells and colonies were screened for the appropriate plasmids. Sequences of the resulting constructs were confirmed by DNA sequencing. --.

Please replace the paragraph at page 38, lines 17-24, beginning "CD5Ig was constructed in identical fashion, using", with the following rewritten paragraph:

-- CD5Ig was constructed in identical fashion, using
CATTGCACAGTCAAGCTTCCATGCCCATGGGTTCTCTGGCCACCTTG (SEQ ID
NO.: 31) as forward primer and
ATCCACAGTGCAGTGATCATTGGATCCTGGCATGTGAC (SEQ ID NO.: 32) as
reverse primer. The PCR product was restriction endonuclease digested and ligated with the
IgCy 1 fragment as described above. The resulting construct (CD5Ig) encoded a mature
protein having an amino acid sequence containing amino acid residues from position 1 to
position 347 of the sequence corresponding to CD5, two amino acids introduced by the
construction procedure (amino acids DQ), followed by DNA encoding amino acids
corresponding to the IgCy 1 hinge region. --.

Please replace the paragraph at page 39, lines 21-30, beginning "Immunostaining and FACS^R Analysis.", with the following rewritten paragraph:

-- Immunostaining and FACS^R Analysis. Transfected CHO or COS cells or activated T cells were analyzed by indirect immunostaining. Before staining, CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with murine mAbs 9.3 (Hansen et al., Immunogenetics 10:247 (1980)) or BB-1 (Yokochi et al., J. Immunol. 128:823 (1981)), or with Ig fusion proteins (all at 10 micrograms/ml in DMEM containing 10% FCS) for 1-2 h at 4 °C. Cells were then washed, and incubated for an additional 0.5-2h at 4 °C with a FITC-conjugated second step reagent (goat anti-mouse Ig serum for murine mAbs, or goat anti-human Ig G γ serum for fusion proteins (Tago, Inc., Burlingame, CA)). Fluorescence was analyzed on a FACS IV^R cell sorter (Becton Dickinson and CO., Mountain View, CA) equipped with a four decade logarithmic amplifier. --.

Please replace the paragraph at page 40, line 28, through page 41, line 5, beginning "mAbs.", with the following rewritten paragraph:

-- mAbs. Murine monoclonal antibodies (mAbs) 9.3 (anti-CD28) and G19-4 (anti-CD3), G3-7 (anti-CD7), BB-1 (anti-B7 antigen) and rat mAb 187.1 (anti-mouse kappa chain) have been described previously (Ledbetter et al., Proc. Natl. Acad. Sci. 84:1384-1388 (1987); Ledbetter et al., Blood 75:1531 (1990); Yokochi et al., supra) and were purified from ascites before use. The hybridoma producing mAb OKT8 was obtained from the ATCC, Rockville, MD, and the mAb was also purified from ascites before use. mAb 4G9 (anti-CD19) was provided by Dr. E. Engleman, Stanford University, Palo Alto, CA). Purified human-mouse chimeric mAb L6 (having human C γ 1 Fc portion) was a gift of Dr. P. Fell and M. Gayle (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA). --.

Please replace the paragraph at page 41, lines 7-15, beginning "Immunostaining and FACS^R Analysis.", with the following rewritten paragraph:

-- Immunostaining and FACS^R Analysis. Prior to staining, COS or CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with mAbs or Ig fusion proteins at 10 micro grams/ml in DMEM containing 10% FBS for 1-2 hr at 4 degrees C. Cells were then washed, and incubated for an additional 0.5-2 hrs at 4 degrees C with FITC-conjugated goat anti-mouse immunoglobulin or with FITC-conjugated goat anti-human IgG serum (both from Tago, Burlingame, CA). When binding of both mAbs and Ig fusion proteins were measured in the same experiment, FITC-conjugated anti-mouse and anti-human second step reagents were mixed together before use. Fluorescence on a total of 10,000 cells was then analyzed by FACS^R. --.

Please replace the paragraph at page 44, lines 10-17, beginning "Binding of CTLA4Ig on B7 Positive CHO cells.", with the following rewritten paragraph:

-- Binding of CTLA4Ig on B7 Positive CHO cells. To further characterize the binding of CTLA4Ig and B7, the binding activity of purified CTLA4Ig on B7⁺ CHO cells and on a lymphoblastoid cell line (PM LCL) was measured in the experiment shown in Figure 5. Amplified transfected CHO cell lines and PM LCLs were incubated with medium only (no addition) or an equivalent concentration of human IgG1-containing proteins (10 micro grams/ml) of CD5Ig, CD28Ig or CTLA4Ig. Binding was detected by FACS^R following addition of FITC-conjugated goat anti-human Ig second step reagents. A total of 10,000 stained cells were analyzed by FACS^R. --.

Please replace the paragraph at page 46, lines 14-19, beginning "Primary mixed lymphocyte reaction (MLR)", with the following rewritten paragraph:

-- Primary mixed lymphocyte reaction (MLR) blasts were stimulated with irradiated T51 lymphoblastoid cells (LC) in the absence or presence of concentrations of murine mAb 9.3 Fab fragments, or B7Ig, CD28Ig or CTLA4Ig immunoglobulin G fusion proteins. Cellular

proliferation was measured by [³H]-thymidine incorporation after 4 days and is expressed as the percentage of incorporation by untreated cultures (21,000 cpm). Figure 9 shows the mean of quadruplicate determinations (SEM \leq 10%).--

Please replace the paragraph at page 48, lines 8-17, beginning "These results demonstrate", with the following rewritten paragraph:

-- These results demonstrate the first expression of a functional protein product of CTLA4 transcripts. CTLA4Ig, a fusion construct containing the extracellular domain of CTLA4 fused to an IgC γ 1 domain, forms a disulfide-linked dimer of M_r approximately 50,000 subunits. Because no interchain disulfides would be predicted to form in the Ig portion of this fusion, it seems likely that cysteines from CTLA4 are involved in disulfide bond formation. The analogous CD28Ig fusion protein (Linsley et al, supra, 1991) also contains interchain disulfide linkage(s). These results suggest that CTLA4 receptor, like CD28 (Hansen et al., Immunogenetics 10:247-260 (1980)), exists on the T cell surface as a disulfide linked homodimer. Although CD28 and CTLA4 are highly homologous proteins, they are immunologically distinct, because the anti-CD28 mAb, mAb 9.3, does not recognize CTLA4 (Figures 4 and 5). --

Please replace the paragraph at page 57, lines 20-23, beginning "In addition, two mutants", with the following rewritten paragraph:

-- In addition, two mutants encoding the residues P103A and Y104A (MYPPAY (SEQ ID NO.: 40) and MYPPPA (SEQ ID NO.: 41), respectively) from the CD28Ig 99MYPPPY104 hexapeptide using CD28Ig as a template were also prepared by the same method. --

Please replace the paragraph at page 57, line 30, through page 58, line 1, beginning "These primers encoded the following sequences:", with the following rewritten paragraph:

-- These primers encoded the following sequences:

CDM8FP:5'-AATACGACTCACTATAGG (SEQ ID NO.: 33)

CDM8RP:5'-CACCACACTGTATTAACC (SEQ ID NO.: 34)

Please replace the paragraph at page 58, line 29, through page 59, line 2, beginning "HS7, HS8, and HS9 constructs", with the following rewritten paragraph:

-- HS7, HS8, and HS9 constructs were prepared by replacing a ~350 base-pair HindIII/HpaI 5' fragment of HS4, HS4-A, and HS4-B, respectively, with the equivalent cDNA fragment similarly digested from HS5 thus introducing the CDR1-like loop of CTLA4 into those hybrids already containing the CTLA4 CDR3-like region. --

Please replace the paragraph at page 62, lines 29-31, beginning "Several versions of the model", with the following rewritten paragraph:

-- Several versions of the model with modified assignments of some residues to β -strands or loops were tested using 3D-profile analysis (Luthy et al., 1992, Nature 336:83-85) in order to improve the initial alignment of the CTLA4 extracellular region sequence with an IGSF variable fold. --

Please replace the paragraph at page 63, lines 12-15, beginning "Regions of sequence conservation", with the following rewritten paragraph:

-- Regions of sequence conservation are scattered throughout the extracellular domains of these proteins with the most rigorous conservation seen in the hexapeptide MYPPPY (SEQ ID NO.: 35) motif located in the CDR3-like loop of both CTLA4 and CD28 (Figure 17). This suggests a probable role for this region in the interaction with a B7 antigen, e.g., B7-1 and B7-2. --

Please replace the TABLE B at page 73, lines 1-45, beginning "TABLE B. Binding of CTLA4 and CD28 monoclonal antibodies", with the following rewritten paragraph:

-- **TABLE B.** Binding of CTLA4 and CD28 monoclonal antibodies to CTLA4Ig and CD28Ig mutant fusion proteins and to CTLA4/CD28Ig hybrid fusion proteins.

	<u>anti-CTLA4 mAbs</u>			<u>anti-CD28 mAb</u>
	7F8	11D4	10A8	9.3
<u>CTLA4Ig MUTANT FUSION PROTEIN</u>				
AYPPPY (SEQ ID NO.: 36) +++		+++	+++	-
MAPPPY (SEQ ID NO.: 37) ++		+	++	-
MYAPPY (SEQ ID NO.: 38) +		-	+	-
MYPAPY (SEQ ID NO.: 39) +++		++++++	+++	-
MYPPAY (SEQ ID NO.: 40) +++		-	+	-
MYPPPA (SEQ ID NO.: 41) +++		++	+++	-
AAPPPY (SEQ ID NO.: 42) +		++	+++	-
<u>CD28Ig MUTANT FUSION PROTEIN</u>				
MYPPAY (SEQ ID NO.: 40) -		-	-	+
MYPPPA (SEQ ID NO.: 41) -		-	-	-
<u>CTLA4/CD28Ig HYBRID FUSION PROTEINS</u>				
HS1	-	-	-	+
HS2	-	-	-	-
HS3	-	-	-	+++
HS4	-	-	-	-
HS5	-	-	-	-
HS6	+	-	-	++
HS4-A	-	-	-	++
HS4-B	-	-	-	+++
HS7	-	+	-	+++
HS8	-	+	-	-
HS9	-	-	-	-
HS10	-	-	-	+
HS11	-	-	-	-
HS12	-	-	-	-
HS13	-	-	-	-
HS14	-	-	-	-
CTLA4Ig	+++	+++	+++	+++
CD28Ig	-	-	-	-

Antibody binding was rated from that seen for wild type protein (+++) to above background (+), and no detectable binding (-).

Please replace the paragraph at page 77, lines 12-17, with the following rewritten paragraph:

-- Experimental data were first fit to a model for a single ligand binding to a single receptor (1-site model, i.e., a simple langmuir system, $A+B \rightleftharpoons AB$), and equilibrium association constants ($K_d = [A] \cdot [B] / [AB]$) were calculated from the equation $R = R_{\max} \cdot C / (K_d + C)$. Subsequently, data were fit to the simplest two-site model of ligand binding (i.e., to a receptor having two non-interacting independent binding sites as described by the equation $R = R_{\max 1} \cdot C / (K_{d1} + C) + R_{\max 2} \cdot C / (K_{d2} + C)$).

Please replace the paragraph at page 84, lines 23-28, beginning "From tyrosine +23 to threonine +30," with the following rewritten paragraph:

-- From tyrosine +23 to threonine +30, a pool of degenerate forward primers were generated having the following sequence:

5' CGA GGC ATC GCT AGC TTT GTG TGT GAG XXK XXK XXK XXK XXK XXK
XXK XXK GAG GTC CGG GTG ACA GT 3' (SEQ ID NO.: 43)

Where X = any of the four nucleotides A, T, G, or C and K = either T, G, or C

Each primer contained a Nhe I restriction enzyme cut site. --

Please replace the paragraph at page 84, lines 30-32, beginning "The reverse primer had the following sequence:", with the following rewritten paragraph:

-- The reverse primer had the following sequence:

5' GGT TGC CGC ACA GAC TTC GGT CAC CTG GCT GTC AGC CTG CCG AAG
CAC TGT CAC CCG GA 3' (SEQ ID NO.: 44) --

Please replace the paragraph at page 86, lines 24-31, beginning "Five mutants were enriched through these 5 rounds of panning," with the following rewritten paragraph:

-- Five mutants were enriched through these 5 rounds of panning.

Mut 9	F-E-P-K-R-G-V-Q (SEQ ID NO.: 45)
Mut 19	W-D-Q-Y-T-G-Y-G (SEQ ID NO.: 46)
Mut 71	W-D-A-Y-R-N-Q-Q (SEQ ID NO.: 47)

Mut 45 Y-D-H-P-Y-D-G-Q (SEQ ID NO.: 48)
Mut 4 W-D-Q-H-V-S-R-R (SEQ ID NO.: 49)
CTLA4 Y-A-S-P-G-K-A-T (SEQ ID NO.: 50)

REMARKS

The changes to the specification update the priority claimed in the subject application, provide SEQ ID NOs and ATCC accession number, and correct typographical errors in the subject application.

The amendments to specification at page 1, lines 5-10 merely update the status of the priority documents for the subject application. The amended priorities are supported by the executed combined Declaration and Power of Attorney submitted with the subject application. Thus, the above amendments do not introduce any new matter, and accordingly their entry is respectfully requested.

The amendments to specification at pages 4, lines 14-16; page 6, lines, 20-25; page 7, lines 12-13; page 7, lines 15-17; page 7, lines 19-21; page 7, lines, 23-25; page 7, lines 27-29; page 8, lines 1-3; page 33, lines 1-21; page 35, lines 19-31; page 37, lines 6-25; page 38, lines 1-9 and 17-24; page 57, lines 20-22 and 33; page 58, line 1; page 63, lines 12-15; page 73, lines 10-21; page 84, lines 23-28 and 30-32; page 86, lines 24-31 are merely to provide SEQ ID NOs in the Detailed Description. A sequence listing, including a paper copy, a computer readable form and a Declaration pursuant to 37 C.F.R. §1.821(f) are submitted herein as Exhibit 2. The amendments to incorporate SEQ ID NOs. do not introduce any new matter and are supported by the disclosure as originally filed. Accordingly, entry of these amendments is respectfully requested.

The amendments to specification, at page 11, lines 1-8, provides the ATCC accession number for the DNA encoding L104EA29YIg which was deposited with the ATCC under the provision of Budapest treaty and appropriately referenced in the originally filed

application. The amendment to incorporate the ATCC accession number for the deposited DNA does not introduce any new matter, and accordingly the entry of the amendment is respectfully requested.

The amendments to specification, at page 9, lines 22-25 merely deletes "Figure 35" to correct a typographical error. The above amendment does not introduce any new matter, and accordingly the entry of the amendment is respectfully requested.

The amendments to specification at page 33, lines 19 and 27; page 36, lines 21-25; page 37, line 27; page 38, lines 6 and 17-24; page 39, line 28; page 41, lines 4 and 12; page 44, line 14; page 46, line 16; and page 48, line 10, incorporates the symbol γ to correct a typographical error for IgC γ 1. The IgC γ 1 is a commonly used abbreviation in the art for IgC gamma1. The term "IgC gamma1" is supported by the specification as originally filed (see pages 11, line 14; 27, line 29, 32, line 29). The above amendment is further supported by U.S. Serial No. 08/228,208 (see page 36, line 27, page 40, line 25), to which this application claims priority. Thus, the above amendments do not introduce any new matter, accordingly their entry is respectfully requested.

The amendments to specification at page 34, line 26, merely incorporates β to correct a typographical error. The support for the amendment can be found on page 34, line 26 of the specification as originally filed. The above amendment does not introduce any new data, accordingly its entry is respectfully requested.

The amendment to specification at page 58, line 29, corrects a typographical error by incorporating \sim . The above amendment is supported by U.S. Serial No. 08/228,208 (page 64, line 18), to which this application claims priority. The amendment to correct the typographical error at page 58, line 29 does not incorporate any new data, and accordingly, its entry is respectfully requested.

The amendment to specification at page 62, line 29, corrects a typographical error by incorporating the symbol β for beta strands. The above amendment is supported by U.S. Serial No. 08/228,208 (page 68, line 27), to which this application claims priority. The above amendment does not introduce any new matter, and accordingly, its entry is respectfully requested.

The amendment to specification at page 77, line 13, corrects a typographical error by incorporating \leftrightarrow . It is commonly known in the art that a 1-site model for a single ligand binding to a single receptor is represented by a simple langmuir system, $A+B \rightleftharpoons AB$. The amendment is further supported by U.S. Serial No. 09/603,825 (page 23, line 10), to which this application claims priority. Thus the above amendment does not introduce any new matter, and accordingly, the entry of the above amendment is respectfully requested.

The changes in the specification do not involve new matter and entry of them is respectfully requested. If a telephone interview would be of assistance in advancing the prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone her at the number provided below.

Applicants: Peter S. 1 et al.
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Filed: July 3, 2000
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No additional fee is deemed necessary in connection with the filing of this Amendment.
If any additional fees are necessary, the Patent Office is authorized to charge any
additional fee to Deposit Account No. 50-0306.

Respectfully submitted,

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MARKED-UP VERSION TO SHOW AMENDMENT OF SPECIFICATION

Please amend the specification at page 1, lines 5-10, to read as follows:

-- This application is a continuation-in-part of U.S. Serial No. 08/228,208, filed April 15, 1994, now U.S. Patent No. 6,090,914, issued July 18, 2000, which was a continuation-in-part of U.S. Serial No. 08/008, 898, filed January 22, 1993, now U.S. Patent No. 5,770,197, issued June 23, 1998, which was a continuation-in part of U.S. Serial No. 07/723,617, filed June 27, 1991, now abandoned; U.S. Serial No. 09/603,825, filed June 26, 2000, which was a continuation-in-part of U.S. Serial No. 09/014,761, filed January 28, 1998, which claims priority of U.S. Serial No. 60/036,594, filed January 31, 1997, now abandoned; and U.S. Serial No. 08/539,436, filed October 5, 1995, now U.S. Patent No. 6,132,992, issued October 17, 2000, [U.S. Serial No. 08/539,436, filed October 19, 1995, and U.S. Serial No. not yet known, filed June 26, 2000, which is a continuation in part of U.S. Serial No. 09/014,761, filed January 28, 1998, which claims priority of U.S. Serial No. 60/036,549, filed January 28, 1997, now abandoned,] the contents of all of which are hereby incorporated by reference, in their entirety, into the present application. --.

Please amend the specification at page 4, lines 14-16, to read as follows:

-- Figure 3: Shows the nucleotide (SEQ ID NO.: 1) and complete amino acid sequence (SEQ ID NO.: 2) encoding human CTLA4 receptor fused to the oncostatin M signal peptide, and including the newly identified N-linked glycosylation site, as described in Example 1, infra. --.

Please amend the specification at page 6, lines 20-25, to read as follows:

-- Figure 17: Depicts sequencing alignment of CD28 and CTLA4 family members. Sequences of human (H) (SEQ ID NO.: 7), mouse (M) (SEQ ID NO.: 5), rat (R) (SEQ ID NO.: 6), and chicken (Ch) CD28 (SEQ ID NO.: 8) are aligned with human (SEQ ID NO.: 3) and mouse CTLA4 (SEQ ID NO.: 4). The signal peptides are underlined with a dashed line. The transmembrane domains are underlined with a solid line. The CDR-analogous regions

are noted. The dark shaded areas highlight complete conservation of residues while the light shaded areas highlight conservative amino acid substitutions in all family members.--

Please amend the specification at page 7, lines 11-12, to read as follows:

-- Figure 22: Depicts the nucleotide (SEQ ID NO.: 9) and amino acid sequence of a CTLA4Ig (SEQ ID NO.: 10) having wildtype extracellular domain of CTLA4. --

Please amend the specification at page 7, lines 15-17, to read as follows:

-- Figure 23: Depicts the nucleotide (SEQ ID NO.: 11) and amino acid (SEQ ID NO.: 12) sequences of L104EIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.--

Please amend the specification at page 7, lines 19-21, to read as follows:

-- Figure 24: Depicts the nucleotide (SEQ ID NO.: 13) and amino acid sequence (SEQ ID NO.: 14) of L104EA29YIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.--

Please amend the specification at page 7, lines 23-25, to read as follows:

-- Figure 25: Depicts the nucleotide (SEQ ID NO.: 15) and amino acid sequences (SEQ ID NO.: 16) of L104EA29LIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124. --

Please amend the specification at page 7, lines 27-29, to read as follows:

-- Figure 26: Depicts the nucleotide (SEQ ID NO.: 17) and amino acid sequences (SEQ ID NO.: 18) of L104EA29TIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124. --

Please amend the specification at page 8, lines 1-3, to read as follows:

-- Figure 27: Depicts the nucleotide (SEQ ID NO.: 19) and amino acid sequences (SEQ ID NO.: 20) of L104EA29Wlg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.--

Please amend the specification at page 8, lines 22-25, to read as follows: Please replace the paragraph at page 9, lines 22-25, with the following rewritten paragraph:

-- Figure 37: [Figure 35] Depicts the results of a FACS assay, showing L104EIg and L104ES25RIg bind CHO cells stably transfected with human CD80 or CD86. A) L104EIg and L104ES25RIg bind to human CD80 CHO-transfected cells; B) L104EIg and L104ES25RIg bind to human CD86 CHO-transfected cells.--

Please amend the specification at page 11, lines 1-8, to read as follows:

-- One embodiment of a soluble CTLA4 has been deposited with the American Type Culture Collection (ATCC) in Manassas, Maryland, under the provisions of the Budapest Treaty on May 31, 1991 and has been accorded ATCC accession number: 68629. ATCC 68629 is DNA encoding *CTLA4*lg. Additionally, the CTLA4Ig-24 CHO cell line has been deposited with the ATCC under the Budapest Treaty on May 31, 1991 and has been accorded accession number ATCC 10762. DNA encoding L104EA29Ylg was submitted for deposit on June 19, 2000 with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209. [The ATCC accession number has not yet been assigned.] The DNA encoding L104EA29Ylg has been accorded ATCC accession number PTA-2104. --

Please amend the specification at page 33, lines 2-21, to read as follows:

-- Because a signal peptide for CTLA4 was not identified in the CTLA4 gene, the N-terminus of the predicted sequence of CTLA4 was fused to the signal peptide of oncostatin M (Malik et al., Mol. and Cell. Biol. 9:2847 (1989)) in two steps using overlapping oligonucleotides. For the first step, the oligonucleotide, CTCAGTCTGGTCCTTGCACTCCTGTTCCAAGCATGGCGAGCATGGCAATGCACG

TGGCCCAGCC (SEQ ID NO.: 21) (which encoded the C terminal 15 amino acids from the oncostatin M signal peptide fused to the N terminal 7 amino acids of CTLA4) was used as forward primer, and TTTGGGCTCCTGATCAGAATCTGGGCACGGTTG (SEQ ID NO.: 22) (encoding amino acid residues 119-125 of the amino acid sequence encoding CTLA4 receptor and containing a Bcl I restriction enzyme site) as reverse primer. The template for this step was cDNA synthesized from 1 micro g of total RNA from H38 cells (an HTLV II infected T cell leukemic cell line provided by Drs. Salahudin and Gallo, NCI, Bethesda, MD). A portion of the PCR product from the first step was reamplified, using an overlapping forward primer, encoding the N terminal portion of the oncostatin M signal peptide and containing a Hind III restriction endonuclease site, CTAGCCACTGAAGCTTCACCAATGGGTGTACTGCTCACACAGAGGACGCTGCTCAGTCTGGTCCTTGCACTC (SEQ ID NO.: 23) and the same reverse primer. The product of the PCR reaction was digested with Hind III and Bcl I and ligated together with a Bcl I/Xba I cleaved cDNA fragment encoding the amino acid sequences corresponding to the hinge, CH2 and CH3 regions of IgC γ 1 into the Hind III/Xba I cleaved expression vector, CDM8 or Hind III/Xba I cleaved expression vector pLN. --.

Please amend the specification at page 33, lines 23-29, to read as follows:

-- A schematic map of the resulting CTLA4Ig fusion construct is shown in Figure 1. Sequences displayed in this figure show the junctions between CTLA4 (upper case letters, unshaded regions) and the signal peptide, SP, of oncostatin M (dark shaded regions), and the hinge, H, of IgC(gamma)1 (stippled regions). The amino acid in parentheses was introduced during construction. Asterisks (*) indicate cysteine to serine mutations introduced in the IgC γ hinge region. The immunoglobulin superfamily V-like domain present in CTLA4 is indicated, as are the CH2 and CH3 domains of IgC(gamma)1. --.

Please amend the specification at page 34, lines 21-27, to read as follows:

-- CTLA4Ig was purified by protein A chromatography from serum-free conditioned supernatants (Figure 2). Concentrations of CTLA4Ig were determined assuming an

extinction coefficient at 280 nm of 1.6 (experimentally determined by amino acid analysis of a solution of known absorbance). Molecular weight standards (lanes 1 and 3, Figure 2) and samples (1 micro grams) of CTLA4Ig (lanes 2 and 4) were subjected to SDS-PAGE (4-12% acrylamide gradient) under non-reducing conditions (- beta ME, lanes 1 and 2) or reducing conditions (+ beta ME, lanes 3 and 4) Proteins were visualized by staining with Coomassie Brilliant Blue. --.

Please amend the specification at page 35, lines 19-31, to read as follows:

-- Because the expression of CTLA4 receptor protein in human lymphoid cells has not been previously reported, it was necessary to locate a source of CTLA4 mRNA. cDNA was reverse transcribed from the total cellular RNA of H38 cells, as noted above, was used for cloning by PCR. For this purpose, the oligonucleotide, GCAATGCACGTGGCCCAGCCTGCTGTGGTAGTG (SEQ ID NO.: 24) (encoding the first 11 amino acids in the predicted coding sequence) was used as a forward primer, and TGATGTAACATGTCTAGATCAATTGATGGGAATAAAATAAGGCTG (SEQ ID NO.: 25) (homologous to the last 8 amino acids in CTLA4 and containing a Xba I site) as reverse primer. The template again was a cDNA synthesized from 1 micro gram RNA from H38 cells. Products of the PCR reaction were cleaved with the restriction endonucleases Nco I and Xba I and the resulting 316 bp product was gel purified. A 340 bp Hind III/Nco I fragment from the CTLA4Ig fusion described above was also gel-purified, and both restriction fragments were ligated into Hind III/Xba I cleaved CDM8 to form OMCTLA. --.

Please amend the specification at page 36, lines 21-25, to read as follows:

-- Receptor-immunoglobulin C gamma (IgC γ) fusion proteins B7Ig and CD28Ig were prepared as described by Linsley et al., in J. Exp. Med. 173:721-730 (1991), incorporated by reference herein. Briefly, DNA encoding amino acid sequences corresponding to the respective receptor protein (e.g. B7) was joined to DNA encoding amino acid sequences

corresponding to the hinge, CH2 and CH3 regions of human IgG1. This was accomplished as follows. --

Please amend the specification at page 37, lines 6-25, to read as follows:

-- Plasmid Construction. Expression plasmids containing cDNA encoding CD28, (as described by Aruffo and Seed, Proc. Natl. Acad. Sci. USA 84:8573 (1987)), were provided by Drs. Aruffo and Seed (Mass General Hospital, Boston, MA). Plasmids containing cDNA encoding CD5, (as described by Aruffo, Cell 61:1303 (1990)), were provided by Dr. Aruffo. Plasmids containing cDNA encoding B7, (as described by Freeman et al., J. Immunol. 143:2714 (1989)), were provided by Dr. Freeman (Dana Farber Cancer Institute, Boston, MA). For initial attempts at expression of soluble forms of CD28 and B7, constructs were made (OMCD28 and OMB7) as described by Linsley et al., J. Exp. Med., supra, in which stop codons were introduced upstream of the transmembrane domains and the native signal peptides were replaced with the signal peptide from oncostatin M (Malik et al., Mol. Cell Biol. 9:2847 (1989)). These were made using synthetic oligonucleotides for reconstruction (OMCD28) or as primers (OMB7) for PCR. OMCD28, is a CD28 cDNA modified for more efficient expression by replacing the signal peptide with the analogous region from oncostatin M. CD28Ig and B7Ig fusion constructs were made in two parts. The 5' portions were made using OMCD28 and OMB7 as templates and the oligonucleotide, CTAGCCACTGAAGCTTCACCATGGGTGTACTGCTCACAC (SEQ ID NO.: 26) (encoding the amino acid sequence corresponding to the oncostatin M signal peptide) as a forward primer, and either TGGCATGGGCTCCTGATCAGGCTTAGAAGGTCCGGGAAA (SEQ ID NO.: 27) or, TTTGGGCTCCTGATCAGGAAAATGCTCTTGCTTGGTTGT (SEQ ID NO.: 28) as reverse primers, respectively. Products of the PCR reactions were cleaved with restriction endonucleases (Hind III and BclII) as sites introduced in the PCR primers and gel purified.--

Please amend the specification at page 37, lines 27, through page 38, line 9, to read as follows:

-- The 3' portion of the fusion constructs corresponding to human IgC γ 1 sequences was made by a coupled reverse transcriptase (from Avian myeloblastosis virus; Life Sciences Associates, Bayport, NY)-PCR reaction using RNA from a myeloma cell line producing human-mouse chimeric mAb L6 (provided by Dr. P. Fell and M. Gayle, Bristol-Myers Squibb Company, Pharmaceutical Research Institute, Seattle, WA) as template. The oligonucleotide,

AAGCAAGAGCATTTCCTGATCAGGAGCCCCAAATCTTCTGACAAAACCTCACA
CATCCCCACCGTCCCCAGCACCTGAACTCCTG (SEQ ID NO.: 29) was used as
forward primer,

CTTCGACCAGTCTAGAAGCATCCTCGTGCGACCGCGAGAGC (SEQ ID NO.: 30)
as reverse primer. Reaction products were cleaved with BclI and XbaI and gel purified. Final constructs were assembled by ligating HindIII/BclI cleaved fragments containing CD28 or B7 sequences together with BclI/XbaI cleaved fragment containing IgC γ 1 sequences into HindIII/XbaI cleaved CDM8. Ligation products were transformed into MC1061/p3 *E. coli* cells and colonies were screened for the appropriate plasmids. Sequences of the resulting constructs were confirmed by DNA sequencing. --

Please amend the specification at page 38, lines 17-24, to read as follows:

-- CD5Ig was constructed in identical fashion, using
CATTGCACAGTCAAGCTTCCATGCCCATGGGTTCTCTGGCCACCTTG (SEQ ID
NO.: 31) as forward primer and

ATCCACAGTGCAAGTATCATTGGATCCTGGCATGTGAC (SEQ ID NO.: 32) as
reverse primer. The PCR product was restriction endonuclease digested and ligated with the
IgC γ 1 fragment as described above. The resulting construct (CD5Ig) encoded a mature
protein having an amino acid sequence containing amino acid residues from position 1 to
position 347 of the sequence corresponding to CD5, two amino acids introduced by the

construction procedure (amino acids DQ), followed by DNA encoding amino acids corresponding to the IgC γ 1 hinge region. --

Please amend the specification at page 39, lines 21-30, to read as follows:

-- Immunostaining and FACS^R Analysis. Transfected CHO or COS cells or activated T cells were analyzed by indirect immunostaining. Before staining, CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with murine mAbs 9.3 (Hansen et al., Immunogenetics 10:247 (1980)) or BB-1 (Yokochi et al., J. Immunol. 128:823 (1981)), or with Ig fusion proteins (all at 10 micrograms/ml in DMEM containing 10% FCS) for 1-2 h at 4 °C. Cells were then washed, and incubated for an additional 0.5-2h at 4 °C with a FITC-conjugated second step reagent (goat anti-mouse Ig serum for murine mAbs, or goat anti-human Ig C γ serum for fusion proteins (Tago, Inc., Burlingame, CA)). Fluorescence was analyzed on a FACS IV^R cell sorter (Becton Dickinson and CO., Mountain View, CA) equipped with a four decade logarithmic amplifier. --

Please amend the specification at page 40, line 8, through page 41, line 5, to read as follows:

-- mAbs. Murine monoclonal antibodies (mAbs) 9.3 (anti-CD28) and G19-4 (anti-CD3), G3-7 (anti-CD7), BB-1 (anti-B7 antigen) and rat mAb 187.1 (anti-mouse kappa chain) have been described previously (Ledbetter et al., Proc. Natl. Acad. Sci. 84:1384-1388 (1987); Ledbetter et al., Blood 75:1531 (1990); Yokochi et al., supra) and were purified from ascites before use. The hybridoma producing mAb OKT8 was obtained from the ATCC, Rockville, MD, and the mAb was also purified from ascites before use. mAb 4G9 (anti-CD19) was provided by Dr. E. Engleman, Stanford University, Palo Alto, CA). Purified human-mouse chimeric mAb L6 (having human C γ 1 Fc portion) was a gift of Dr. P. Fell and M. Gayle (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA). --

Please amend the specification at page 41, lines 7-15, to read as follows:

-- Immunostaining and FACS^R Analysis. Prior to staining, COS or CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with mAbs or Ig fusion proteins at 10 micro grams/ml in DMEM containing 10% FBS for 1-2 hr at 4 degrees C. Cells were then washed, and incubated for an additional 0.5-2 hrs at 4 degrees C with FITC-conjugated goat anti-mouse immunoglobulin or with FITC-conjugated goat anti-human Ig G₁ serum (both from Tago, Burlingame, CA). When binding of both mAbs and Ig fusion proteins were measured in the same experiment, FITC-conjugated anti-mouse and anti-human second step reagents were mixed together before use. Fluorescence on a total of 10,000 cells was then analyzed by FACS^R. --.

Please amend the specification at page 44, lines 10-17, to read as follows:

-- Binding of CTLA4Ig on B7 Positive CHO cells. To further characterize the binding of CTLA4Ig and B7, the binding activity of purified CTLA4Ig on B7⁺ CHO cells and on a lymphoblastoid cell line (PM LCL) was measured in the experiment shown in Figure 5. Amplified transfected CHO cell lines and PM LCLs were incubated with medium only (no addition) or an equivalent concentration of human IgG₁-containing proteins (10 micro grams/ml) of CD5Ig, CD28Ig or CTLA4Ig. Binding was detected by FACS^R following addition of FITC-conjugated goat anti-human Ig second step reagents. A total of 10,000 stained cells were analyzed by FACS^R. --.

Please amend the specification at page 46, lines 14-19, to read as follows:

-- Primary mixed lymphocyte reaction (MLR) blasts were stimulated with irradiated T51 lymphoblastoid cells (LC) in the absence or presence of concentrations of murine mAb 9.3 Fab fragments, or B7Ig, CD28Ig or CTLA4Ig immunoglobulin G₁ fusion proteins. Cellular proliferation was measured by [³H]-thymidine incorporation after 4 days and is expressed as the percentage of incorporation by untreated cultures (21,000 cpm). Figure 9 shows the mean of quadruplicate determinations (SEM \leq 10%).--.

Please amend the specification at page 48, lines 8-17, to read as follows:

-- These results demonstrate the first expression of a functional protein product of CTLA4 transcripts. CTLA4Ig, a fusion construct containing the extracellular domain of CTLA4 fused to an IgC γ 1 domain, forms a disulfide-linked dimer of M $_r$ approximately 50,000 subunits. Because no interchain disulfides would be predicted to form in the Ig portion of this fusion, it seems likely that cysteines from CTLA4 are involved in disulfide bond formation. The analogous CD28Ig fusion protein (Linsley et al, supra, 1991) also contains interchain disulfide linkage(s). These results suggest that CTLA4 receptor, like CD28 (Hansen et al., Immunogenetics 10:247-260 (1980)), exists on the T cell surface as a disulfide linked homodimer. Although CD28 and CTLA4 are highly homologous proteins, they are immunologically distinct, because the anti-CD28 mAb, mAb 9.3, does not recognize CTLA4 (Figures 4 and 5). --

Please amend the specification at page 57, lines 20-23, to read as follows:

-- In addition, two mutants encoding the residues P103A and Y104A (MYPPAY (SEQ ID NO.: 40) and MYPPPA (SEQ ID NO.: 41), respectively) from the CD28Ig 99MYPPPY104 hexapeptide using CD28Ig as a template were also prepared by the same method. --.

Please amend the specification at page 57, lines 30, through page 58, line 1, to read as follows:

-- These primers encoded the following sequences:

CDM8FP:5'-AATACGACTCACTATAGG (SEQ ID NO.: 33)

CDM8RP:5'-CACCACACTGTATTAACC (SEQ ID NO.: 34)

Please amend the specification at page 58, line 29, through page 59, line 2, to read as follows:

-- HS7, HS8, and HS9 constructs were prepared by replacing a ~350 base-pair HindIII/HpaI 5' fragment of HS4, HS4-A, and HS4-B, respectively, with the equivalent cDNA fragment similarly digested from HS5 thus introducing the CDR1-like loop of CTLA4 into those hybrids already containing the CTLA4 CDR3-like region. --.

Please amend the specification at page 62, lines 29-31, to read as follows:

-- Several versions of the model with modified assignments of some residues to β -strands or loops were tested using 3D-profile analysis (Luthy et al., 1992, Nature 336:83-85) in order to improve the initial alignment of the CTLA4 extracellular region sequence with an IGSF variable fold. --

Please amend the specification at page 63, lines 12-15, to read as follows:

-- Regions of sequence conservation are scattered throughout the extracellular domains of these proteins with the most rigorous conservation seen in the hexapeptide MYPPPY (SEQ ID NO.: 35) motif located in the CDR3-like loop of both CTLA4 and CD28 (Figure 17). This suggests a probable role for this region in the interaction with a B7 antigen, e.g., B7-1 and B7-2. --

Please amend the specification at page 73, lines 1-45, to read as follows:

-- **TABLE B.** Binding of CTLA4 and CD28 monoclonal antibodies to CTLA4Ig and CD28Ig mutant fusion proteins and to CTLA4/CD28Ig hybrid fusion proteins.

	<u>anti-CTLA4 mAbs</u>		<u>anti-CD28 mAb</u>
	7F8	11D4	10A8
			9.3
<u>CTLA4Ig MUTANT FUSION PROTEIN</u>			
AYPPPY (SEQ ID NO.: 36) +++		+++	+++
MAPPPY (SEQ ID NO.: 37) ++		+	++
MYAPPY (SEQ ID NO.: 38) +		-	+
MYPAPY (SEQ ID NO.: 39) +++		++++++	+++
MYPPAY (SEQ ID NO.: 40) +++		-	+

MYPPPA (SEQ ID NO.: 41)	+++	++	+++	-
AAPPPY (SEQ ID NO.: 42)	+	++	+++	-

CD28Ig MUTANT FUSION PROTEIN

MYPPAY (SEQ ID NO.: 40)	-	-	-	-
MYPPPA (SEQ ID NO.: 41)	-	-	-	+

CTLA4/CD28Ig HYBRID FUSION PROTEINS

HS1	-	-	-	-
HS2	-	-	-	+
HS3	-	-	-	-
HS4	-	-	-	+++
HS5	-	-	-	-
HS6	+	-	-	-
HS4-A	-	-	-	++
HS4-B	-	-	-	++
HS7	-	-	-	+++
HS8	-	+	-	+++
HS9	-	+	-	-
HS10	-	-	-	-
HS11	-	-	-	+
HS12	-	-	-	-
HS13	-	-	-	-
HS14	-	-	-	-
CTLA4Ig	+++	+++	+++	-
CD28Ig	-	-	-	+++

Antibody binding was rated from that seen for wild type protein (+++) to above background (+), and no detectable binding (-). --.

Please amend the specification at page 77, lines 12-17, to read as follows:

-- Experimental data were first fit to a model for a single ligand binding to a single receptor (1-site model, i.e., a simple langmuir system, $A+B \rightleftharpoons AB$), and equilibrium association constants ($K_d=[A] \cdot [B]/[AB]$) were calculated from the equation $R=R_{max} \cdot C/(K_d+C)$. Subsequently, data were fit to the simplest two-site model of ligand binding (i.e., to a receptor having two non-interacting independent binding sites as described by the equation $R=R_{max1} \cdot C/(K_{d1}+C)+R_{max2} \cdot C/(K_{d2}+C)$).--.

Please amend the specification at page 84, lines 23-28, to read as follows:

-- From tyrosine +23 to threonine +30, a pool of degenerate forward primers were generated having the following sequence:

5' CGA GGC ATC GCT AGC TTT GTG TGT GAG XXK XXK XXK XXK XXK
XXK XXK GAG GTC CGG GTG ACA GT 3' (SEQ ID NO.: 43)

Where X = any of the four nucleotides A, T, G, or C and K = either T, G, or C

Each primer contained a Nhe I restriction enzyme cut site. --.

Please amend the specification at page 84, lines 30-32, to read as follows:

-- The reverse primer had the following sequence:

5' GGT TGC CGC ACA GAC TTC GGT CAC CTG GCT GTC AGC CTG CCG AAG
CAC TGT CAC CCG GA 3' (SEQ ID NO.: 44) --.

Please amend the specification at page 86, lines 24-31, to read as follows:

-- Five mutants were enriched through these 5 rounds of panning.

Mut 9	F-E-P-K-R-G-V-Q (SEQ ID NO.: 45)
Mut 19	W-D-Q-Y-T-G-Y-G (SEQ ID NO.: 46)
Mut 71	W-D-A-Y-R-N-Q-Q (SEQ ID NO.: 47)
Mut 45	Y-D-H-P-Y-D-G-Q (SEQ ID NO.: 48)
Mut 4	W-D-Q-H-V-S-R-R (SEQ ID NO.: 49)
CTLA4	Y-A-S-P-G-K-A-T (SEQ ID NO.: 50)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Peter S. Linsley et al.

Serial No.: 09/609,915

Examiner: E. M. Lazar-Wesley, Ph.D.

Filed: July 3, 2000

Group Art Unit: 1646

For: SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

35 N. Arroyo Pkwy., Suite 60
Pasadena, California 91103
April 15, 2002

Honorable Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

**COMMUNICATION IN RESPONSE TO A NOTICE TO COMPLY WITH
REQUIREMENTS OF 37 CFR 1.821-1.825 FOR PATENT APPLICATIONS
CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE
DISCLOSURES DATED MARCH 15, 2002**

This communication is submitted in response to an Office Communication dated March 15, 2002, to comply with requirements of 37 C.F.R. 1.821 through 1.825 for sequence listing, in connection with the above-identified application. A one (1) month period for reply was set, making April 15, 2002, the deadline for filing a response to the Notice. Accordingly, this Response is being timely filed. A copy of the above-referenced Notice is submitted herein as Exhibit 1.

In response to the Office Communication, the applicants herein submit a sequence listing including an original paper copy, a computer readable copy, and a Declaration under 37 C.F.R. §1.821(f) stating that the computer readable copy of the sequence listing is identical to the paper

Applicants: Peter S. Lins., et al.
U.S. Serial No. 09/609,915
Filed: July 3, 2000
Page 2

copy (Exhibit 2). The sequence listing contains no new matter and is supported by the specification as originally filed. Accordingly, entry of this amendment is respectfully requested.

No additional fee is deemed necessary in connection with the filing of this Communication. However, if any additional fee is necessary, the Patent Office is authorized to charge the additional fee to Deposit Account No. 50-0306.

Respectfully submitted,

Sarah B. Adriano

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Registration No. 34,470
SaraLynn Mandel
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35 N. Arroyo Parkway
Pasadena, California 91103
(626) 395-7801
Customer Number: 26941

EXHIBIT 1

Copy of Notice to Comply
with Requirements for
Patent Applications
Containing Nucleotide
Sequence and/or Amino
Acid Sequence Disclosures

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING
NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 CFR 1.821 - 1.825 for the following reason(s):

☒ 1. This application clearly fails to comply with the requirements of 37 CFR 1.821 - 1.825. Applicant's attention is directed to these regulations, published at 1114 OG 29, May 15, 1990 and at 55 FR 18230, May 1, 1990.

☒ 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 CFR 1.821(c).

☐ 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 CFR 1.821(e).

☐ 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 CFR 1.822 and/or 1.823, as indicated on the attached copy of the marked-up "Raw Sequence Listing."

☐ 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A substitute computer readable form must be submitted as required by 37 CFR 1.825(d).

☐ 6. The paper copy of the "Sequence Listing" is not the same as the computer readable form of the "Sequence Listing" as required by 37 CFR 1.821(e).

☒ 7. Applicants should follow the format of the attached sample
Other: ~~statement if they request that the CRF filed in the parent~~
Applicant must provide: ~~application should be used to create a CRF~~
~~in this application.~~

☒ An initial or substitute computer readable form (CRF) copy of the "Sequence Listing"

☒ An initial or substitute paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification

☒ A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 CFR 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d)

For questions regarding compliance with these requirements, please contact

For Rules Interpretation, call (703) 308-1123
For CRF submission help, call (703) 308-4212
For PatentIn software help, call (703) 557-0400

Please return a copy of this notice with your response.

EXHIBIT 2

Sequence Listing in paper
copy and Declaration
Pursuant to 37 C.F.R.
§1.821(f)

SEQUENCE LISTING

<110> Linsley, Peter S
Ledbetter, Jeffrey A
Bajorath, Jurgen
Peach, Robert J
Brady, William
Wallace, Philip
Damle, Nitin K

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 ggcacgcta gctttgtgtg tgagtatgca tctccaggca aagccactga ggtccgggtg 180
 acagtgcctc ggcaggctga cagccagggtg actgaagtct gtgcggcaac ctacatgatg 240
 gggaatgagt tgaccttcct agatgatccc atctgcacgg gcacctccag tggaaatcaa 300
 gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg 360
 gagctcatgt acccaccgcc atactacctg ggcataggca acggaaccca gatttatgta 420
 attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480
 acatccccac cgtccccagc acctgaactc ctgggtggat cgtcagtctt cctcttcccc 540
 ccaaaaccca aggacacct catgatctcc cggaccctg aggtcacatg cgtggtggtg 600
 gacgtgagcc acgaagaccc tgaggtaag ttcaactgg acgtggacgg cgtggagggtg 660
 cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg ggtggtcagc 720
 gtcctcaccg tcttgacca ggactggctg aatggcaagg agtacaagtg caaggctctc 780
 aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840
 gaaccacagg tgtacacct gcccccattc cgggatgagc tgaccaagaa ccaggtcagc 900
 ctgacctgcc tggtaaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960
 gggcagccgg agaacaacta caagaccacg cctcccgctg tggactccga cggctccttc 1020
 ttcctctaca gcaagctcac cgtggacaag agcagggtggc agcaggggaa cgtcttctca 1080
 tgctccgtga tgcattgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140
 ccgggtaaat ga 1152

<210> 10
 <211> 383
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: CTLA4Ig

<400> 10

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
35 40 45

Tyr Ala Ser Pro Gly Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg
50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Leu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
 260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
 275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
 340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375 380

<210> 11
 <211> 1152
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EIg

<400> 11
 atgggtgtac tgctcacaca gaggacgctg ctcagtctgg tcttgcaact cctgtttcca 60
 agcatggcga gcatggcaat gcacgtggcc cagcctgctg tggactggc cagcagccga 120
 ggcacgcta gctttgtgtg tgagtatgca tctccaggca aagccactga ggtccgggtg 180
 acagtgttc ggcaggctga cagccagggtg actgaagtct gtgcggcaac ctacatgatg 240

gggaatgagt tgaccttcct agatgattcc atctgcacgg gcacctccag tggaaatcaa 300
 gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg 360
 gagctcatgt acccacggcc atactacgag ggcataggca acggaacca gatttatgta 420
 attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480
 acatccccac cgtccccagc acctgaactc ctggggggat cgtcagtctt cctcttcccc 540
 ccaaaacca aggacacct catgatctcc cggacctctg aggtcacatg cgtggtggtg 600
 gacgtgagcc acgaagacc tgaggtcaag ttcaactggt acgtggacgg cgtggagggtg 660
 cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 720
 gtcttcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 780
 aacaaagccc tcccagcccc catcgagaaa accatctcca agccaaagg gcagccccga 840
 gaaccacagg tgtacacct gccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900
 ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960
 gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc 1020
 ttctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080
 tgctccgtga tgcattgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140
 ccgggtaaat ga 1152

<210> 12
 <211> 383
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EIg

<400> 12

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
 1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
 20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
 35 40 45

Tyr Ala Ser Pro Gly Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg

50

55

60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
 340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375 380

<210> 13
 <211> 1152
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EA29YIg

<400> 13
 atgggtgtac tgctcacaca gaggacgctg ctcagtctgg tccttgcaact cctgtttcca 60
 agcatggcga gcatggcaat gcacgtggcc cagcctgctg tgggtactggc cagcagccga 120
 ggcacgccta gctttgtgtg tgagtatgca tctccaggca aatatactga ggtccgggtg 180
 acagtgtctc ggcaggctga cagccagggt actgaagtct gtgcggcaac ctacatgatg 240
 gggaatgagt tgaccttcct agatgattcc atctgcacgg gcacctccag tggaaatcaa 300
 gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg 360
 gagtcatgt acccacgcc atactacgag ggcataaggca acggaacca gatttatgta 420
 attgatccag aaccgtgcc agattctgat caggagccca aatcttctga caaaactcac 480
 acatccccac cgtccccagc acctgaactc ctgggggggat cgtcagtctt cctcttcccc 540
 ccaaaacca aggacacct catgatctcc cggaccctg aggtcacatg cgtgggtgtg 600
 gacgtgagcc acgaagacc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg 660

cataatgcc aagacaaaagcc ggggaggag cagtacaaca gcacgtaccg tgtggtcagc 720
gtcctcaccg tectgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 780
aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840
gaaccacagg tgtacacctt gccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900
ctgacctgcc tggtaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960
gggcagccgg agaacaacta caagaccacg cctcccgctg tggactccga cggctccttc 1020
ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080
tgctccgtga tgcattaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140
ccgggtaaat ga 1152

<210> 14
<211> 383
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EA29YIg

<400> 14

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
35 40 45

Tyr Ala Ser Pro Gly Lys Tyr Thr Glu Val Arg Val Thr Val Leu Arg
50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg

340

345

350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375 380

<210> 15
 <211> 1152
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EA29L19

<400> 15
 atgggtgtac tgctcacaca gaggacgtg ctcaagtctgg tccttgcaact cctgtttcca 60
 agcatggcga gcatggcaat gcacgtggcc cagcctgctg tggtaactggc cagcagccga 120
 ggcatcgcta gctttgtgtg tgagtatgca tctccaggca aattgactga ggtccgggtg 180
 acagtgttcc ggcaggctga cagccagggtg actgaagtct gtgcggcaac ctacatgatg 240
 gggaatgagt tgaccttccct agatgattcc atctgcacgg gcacctccag tggaaatcaa 300
 gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg 360
 gagctcatgt acccaccgcc atactacgag ggcataaggca acggaaccca gatttatgta 420
 attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480
 acatccccac cgtccccagc acctgaactc ctggggggat cgtcagtctt cctcttcccc 540
 ccaaaaccca aggacacctt catgatctcc cggaccctg aggtcacatg cgtgggtggtg 600
 gacgtgagcc acgaagaccc tgagggtcaag ttcaactggt acgtggacgg cgtggaggtg 660
 cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 720
 gtcttcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 780
 aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840
 gaaccacagg tgtacaccct gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900
 ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960
 gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc 1020
 ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080
 tgctccgtga tgcattgagg tctgcacaac cactacacgc agaagagcct ctccctgtct 1140

ccgggtaaat ga

<210> 16
 <211> 383
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EA29LIg

<400> 16

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
 1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
 20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
 35 40 45

Tyr Ala Ser Pro Gly Lys Leu Thr Glu Val Arg Val Thr Val Leu Arg
 50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
 65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
 85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
 100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
 115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
 130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
 145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
 165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
 210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
 260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
 275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
 340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375 380

<210> 17
 <211> 1152
 <212> DNA
 <213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EA29TIg

<400> 17
atgggtgtac tgctcacaca gaggacgctg ctcagtcttg tccttgcaact cctgtttcca 60
agcatggcga gcatggcaat gcacgtggcc cagcctgctg tggtaactggc cagcagccga 120
ggcatcgcta gctttgtgtg tgagtatgca tctcdaggca aaactactga ggtccgggtg 180
acagtgcttc ggcaggetga cagccaggtg actgaagtct gtgcggcaac ctacatgatg 240
gggaatgagt tgaccttctt agatgattcc atctgcacgg gcacctccag tggaaatcaa 300
gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg 360
gagctcatgt acccaccgcc atactacgag ggcataggca acggaaccca gatttatgta 420
attgatccag aaccgtgccc agattctgat caggagccca aatctttctga caaaactcac 480
acatccccac cgtccccagc acctgaactc ctgggggggat cgtcagtctt cctcttcccc 540
ccaaaaccca aggacaccct catgatctcc eggacccttg aggtcacatg cgtggtggtg 600
gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg 660
cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 720
gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 780
aaciaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840
gaaccacagg tgtacaccct gccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900
ctgacctgcc tggtaaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960
gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc 1020
ttctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080
tgctccgtga tgcattgagc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140
ccgggtaaat ga 1152

<210> 18
<211> 383
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EA29TIg

<400> 18
Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala

1 5 10 15
 Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
 20 25 30
 Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
 35 40 45
 Tyr Ala Ser Pro Gly Lys Thr Thr Glu Val Arg Val Thr Val Leu Arg
 50 55 60
 Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
 65 70 75 80
 Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
 85 90 95
 Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
 100 105 110
 Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
 115 120 125
 Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
 130 135 140
 Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
 145 150 155 160
 Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
 165 170 175
 Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 180 185 190
 Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 195 200 205
 Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
 210 215 220
 Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
 260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
 275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
 340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375 380

<210> 19
 <211> 1152
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EA29W1g

<400> 19
 atgggtgtac tgctcacaca gaggacgctg ctcagtctgg tccttgcaact cctgtttcca 60
 agcatggcga gcatggcaat gcacgtggcc cagcctgctg tggactggc cagcagccga 120
 ggcacgcta gctttgtgtg tgagtatgca tctccaggca aatggactga ggtccgggtg 180
 acagtgttc ggcaggctga cagccagggtg actgaagtct gtgcggcaac ctacatgatg 240
 gggaatgagt tgaccttct agatgattcc atctgcacgg gcacctccag tggaaatcaa 300

gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg 360
 gagctcatgt acccaccgcc atactacgag ggcataggca acggaaccca gatttatgta 420
 attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480
 acatccccac cgtccccagc acctgaactc ctggggggat cgtcagtctt cctcttcccc 540
 ccaaaaccca aggacacct catgatctcc cggaccctg aggtcacatg cgtgggtgtg 600
 gacgtgagcc acgaagaccc tgaggtcaag ttcaactgg acgtggacgg cgtggaggtg 660
 cataatgcc aagacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 720
 gtcctcacgc tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 780
 aacaaagccc tccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840
 gaaccacagg tgtacacct gccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900
 ctgacctgcc tggtaaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960
 gggcagccgg agaacaacta caagaccag cctcccgtgc tggactccga cggctccttc 1020
 ttctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080
 tgctccgtga tgcattgagg tctgcacaac cactacacgc agaagagcct ctccctgtct 1140
 ccgggtaaat ga 1152

<210> 20
 <211> 383
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EA29W1g

<400> 20

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
 1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
 20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
 35 40 45

Tyr Ala Ser Pro Gly Lys Trp Thr Glu Val Arg Val Thr Val Leu Arg
 50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
 65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
 85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
 100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
 115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
 130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
 145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
 165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
 210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
 260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
 275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu

290

295

300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
 340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
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His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375 380

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<210> 23
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 <212> DNA
 <213> Homo sapiens

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 gtccttgac tc 72

<210> 24
 <211> 33
 <212> DNA

<213> Homo sapiens

<400> 24
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33

<210> 25
<211> 45
<212> DNA
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<400> 25
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<210> 26
<211> 39
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<223> Description of Artificial Sequence: Oncostatin M signal peptide forward primer

<400> 26
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39

<210> 27
<211> 39
<212> DNA
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<400> 27
tggcatgggc tcctgatcag gcttagaagg tccgggaaa

39

<210> 28
<211> 39
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<220>
<223> Description of Artificial Sequence: Oncostatin M signal peptide reverse primer

<400> 28
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39

<210> 29
<211> 84
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<223> Description of Artificial Sequence: Human IgCgamma1 forward prime
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ccgtccccag cacctgaact cctg

<210> 30

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<223> Description of Artificial Sequence: Human IgCgamma1 reverse prime
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<210> 31

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<223> Description of Artificial Sequence: CD5Ig forward primer

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<223> Description of Artificial Sequence: CD5Ig reverse primer

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<212> DNA

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<223> Description of Artificial Sequence: CDM8 forward primer

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18

<210> 34
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<400> 34
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18

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<400> 35

Met Tyr Pro Pro Pro Tyr
1 5

<210> 36
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<223> Description of Artificial Sequence: CTLA4Ig mutant fusion protein

<400> 36

Ala Tyr Pro Pro Pro Tyr
1 5

<210> 37
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<223> Description of Artificial Sequence: CTLA4Ig mutant fusion protein

<400> 37

Met Ala Pro Pro Pro Tyr
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<210> 38
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<400> 38

Met Tyr Ala Pro Pro Tyr
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<220>
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<400> 39

Met Tyr Pro Ala Pro Tyr
1 5

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Met Tyr Pro Pro Ala Tyr
1 5

<210> 41
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<400> 41

Met Tyr Pro Pro Pro Ala
1 5

<210> 42
<211> 6
<212> PRT
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<400> 42

Ala Ala Pro Pro Pro Tyr
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<210> 43
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<210> 44
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Phage display reverse primer

<400> 44
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<210> 45
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4 mutant

<400> 45

Phe Glu Pro Lys Arg Gly Val Gln
1 5

<210> 46
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4 mutant

<400> 46

Trp Asp Gln Tyr Thr Gly Tyr Gly
1 5

<210> 47
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4 mutant

<400> 47

Trp Asp Ala Tyr Arg Asn Gln Gln
1 5

<210> 48

<211> 8

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: CTLA4 mutant

<400> 48

Tyr Asp His Pro Tyr Asp Gly Gln
1 5

<210> 49

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: CTLA4 mutant

<400> 49

Trp Asp Gln His Val Ser Arg Arg
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<210> 50

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: CTLA4

<400> 50

Tyr Ala Ser Pro Gly Lys Ala Thr
1 5

SEQUENCE LISTING

<110> Linsley, Peter S.
Ledbetter, Jeffrey A.
Bajorath, Jurgen
Peach, Robert J.
Brady, William
Wallace, Philip
Damle, Nitin K

<120> SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

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<150> 07/723,617

<151> 1991-06-27

<150> 08/008,898

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ggcatcgcca gctttgtgtg tgagtatgca tctccaggca aagccactga ggtccgggtg	180
acagtgtctc ggcaggctga cagccagggtg actgaagtct gtgcggcaac ctacatgatg	240
gggaatgagt tgaccttct agatgattcc atctgcacgg gcacctccag tggaaatcaa	300

gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg 360
 gagctcatgt acccaccgcc atactacctg ggcataaggca acggaaccca gatttatgta 420
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 tcgggggtgt ttttttatag ctttctctct acagctgttt ctttgagcaa aatgctaaag 540
 aaaagaagcc ctcttacaac aggggtctat gtgaaaatgc ccccaacaga gccagaatgt 600
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Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
 20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
 35 40 45

Tyr Ala Ser Pro Gly Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg
 50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
 65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
 85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
 100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
 115 120 125

Tyr Leu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
 130 135 140

Pro Cys Pro Asp Ser Asp Phe Leu Leu Trp Ile Leu Ala Ala Val Ser
 145 150 155 160

Ser Gly Leu Phe Phe Tyr Ser Phe Leu Leu Thr Ala Val Ser Leu Ser
 165 170 175

Lys Met Leu Lys Lys Arg Ser Pro Leu Thr Thr Gly Val Tyr Val Lys
 180 185 190

Met Pro Pro Thr Glu Pro Glu Cys Glu Lys Gln Phe Gln Pro Tyr Phe
 195 200 205

Ile Pro Ile Asn
 210

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 <212> PRT
 <213> Homo sapiens
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Ala Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro
 20 25 30

Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
 35 40 45

Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
 50 55 60

Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
 65 70 75 80

Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr
 85 90 95

Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
 100 105 110

Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
 115 120 125

Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly
130 135 140

Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser
145 150 155 160

Asp Phe Leu Leu Trp Ile Leu Ala Ala Val Ser Ser Gly Leu Phe Phe
155 170 175

Tyr Ser Phe Leu Leu Thr Ala Val Ser Leu Ser Lys Met Leu Lys Lys
180 185 190

Arg Ser Pro Leu Thr Thr Gly Val Tyr Val Lys Met Pro Pro Thr Glu
195 200 205

Pro Glu Cys Glu Lys Gln Phe Gln Pro Tyr Phe Ile Pro Ile Asn
210 215 220

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Ser Arg Thr Trp Pro Phe Val Ala Leu Leu Thr Leu Leu Phe Ile Pro
20 25 30

Val Phe Ser Glu Ala Ile Gln Val Thr Gln Pro Ser Val Tyr Leu Ala
35 40 45

Ser Ser His Gly Tyr Ala Ser Phe Pro Cys Glu Tyr Ser Pro Ser His
50 55 60

Asn Thr Asp Glu Val Arg Val Thr Val Leu Arg Gln Thr Asn Asp Gln
65 70 75 80

Met Thr Glu Val Cys Ala Thr Thr Phe Thr Glu Lys Asn Thr Val Gly
85 90 95

Phe Leu Asp Tyr Pro Phe Cys Ser Gly Thr Phe Asn Glu Ser Arg Val
100 105 110

Asn Leu Thr Ile Gln Gly Leu Arg Ala Val Asp Thr Gly Leu Tyr Leu
115 120 125

Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Phe Val Gly Met Gly
130 135 140

Asn Gly Thr Gln Ile Tyr Tyr Ile Asp Pro Glu Pro Cys Pro Asp Ser
145 150 155 160

Asp Phe Leu Leu Trp Ile Leu Tyr Ala Val Ser Leu Gly Leu Phe Phe
165 170 175

Tyr Ser Phe Leu Val Ser Ala Val Ser Leu Ser Lys Met Leu Lys Lys
180 185 190

Arg Ser Pro Leu Thr Thr Gly Val Tyr Val Lys Met Pro Pro Thr Glu
195 200 205

Pro Glu Cys Glu Lys Gln Phe Gln Pro Tyr Phe Ile Pro Ile Asn
210 215 220

<210> 5
<211> 218
<212> PRT
<213> Mus musculus

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20 25 30

Asp Ser Asn Glu Val Ser Leu Ser Cys Arg Tyr Ser Tyr Asn Leu Leu
35 40 45

Ala Lys Glu Phe Arg Ala Ser Leu Tyr Lys Gly Val Asn Ser Asp Val
50 55 60

Glu Val Cys Val Gly Asn Gly Asn Phe Thr Tyr Gln Pro Gln Phe Arg
65 70 75 80

Ser Asn Ala Glu Phe Asn Cys Asp Gly Asp Phe Asp Asn Glu Thr Val
85 90 95

Thr Phe Arg Leu Trp Asn Leu His Val Asn His Thr Asp Ile Tyr Phe
100 105 110

Cys Lys Ile Glu Phe Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Arg
115 120 125

Ser Asn Gly Thr Ile Ile His Ile Lys Glu Lys His Leu Cys His Thr
130 135 140

Gln Ser Ser Pro Lys Leu Phe Trp Ala Leu Tyr Val Val Ala Gly Val
145 150 155 160

Leu Phe Cys Tyr Gly Leu Leu Val Thr Val Ala Leu Cys Val Ile Trp
165 170 175

Thr Asn Ser Arg Arg Asn Arg Leu Leu Gln Val Thr Tyr Met Asn Met
180 185 190

Thr Pro Arg Arg Pro Gly Leu Thr Arg Lys Pro Tyr Gln Pro Tyr Ala
195 200 205

Pro Ala Arg Asp Phe Ala Ala Tyr Arg Pro
210 215

<210> 6
<211> 218
<212> PRT
<213> Rattus norvegicus

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Met Thr Leu Arg Leu Leu Phe Leu Ala Leu Ser Phe Phe Ser Val Gln
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Val Thr Glu Asn Lys Ile Leu Val Lys Gln Ser Pro Leu Leu Val Tyr
20 25 30

Asp Asn Asn Glu Val Ser Leu Ser Cys Arg Tyr Ser Tyr Asn Leu Leu
35 40 45

Ala Lys Glu Phe Arg Ala Ser Leu Tyr Lys Gly Val Asn Ser Asp Val
50 55 60

Glu Val Cys Val Gly Asn Gly Asn Phe Thr Tyr Gln Pro Gln Phe Arg
65 70 75 80

Pro Asn Val Gly Phe Asn Cys Asp Gly Asn Phe Asp Asn Glu Thr Val
85 90 95

Thr Phe Arg Leu Trp Asn Leu Asp Val Asn His Thr Asp Ile Tyr Phe
100 105 110

Cys Lys Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys
115 120 125

Ser Asn Gly Thr Ile Ile His Ile Lys Glu Lys His Leu Cys His Ala
130 135 140

Gln Thr Ser Pro Lys Leu Phe Trp Pro Leu Val Val Val Ala Gly Val
145 150 155 160

Leu Leu Cys Tyr Gly Leu Leu Tyr Thr Val Thr Leu Cys Ile Ile Trp
165 170 175

Thr Asn Ser Arg Arg Asn Arg Leu Leu Gln Ser Asp Tyr Met Asn Met
180 185 190

Thr Pro Arg Arg Leu Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala
195 200 205

Pro Ala Arg Asp Phe Ala Ala Tyr Arg Pro
210 215

<210> 7
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<212> PRT
<213> Homo sapiens

<400> 7

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Thr Gly Asn Lys Ile Leu Val Lys Gln Ser Pro Met Leu Val Ala Tyr
20 25 30

Asp Asn Ala Tyr Asn Leu Ser Cys Lys Tyr Ser Tyr Asn Leu Phe Ser
 35 40 45

Arg Glu Phe Arg Ala Ser Leu His Lys Gly Leu Asp Ser Ala Val Glu
 50 55 60

Val Cys Val Val Tyr Gly Asn Tyr Ser Gln Gln Leu Gln Val Tyr Ser
 65 70 75 80

Lys Thr Gly Phe Asn Cys Asp Gly Lys Leu Gly Asn Glu Ser Val Thr
 85 90 95

Phe Tyr Leu Gln Asn Leu Tyr Val Asn Gln Thr Asp Ile Tyr Phe Cys
 100 105 110

Lys Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys Ser
 115 120 125

Asn Gly Thr Ile Ile His Val Lys Gly Lys His Leu Cys Pro Ser Pro
 130 135 140

Leu Phe Pro Gly Pro Ser Lys Pro Phe Trp Val Leu Val Val Val Gly
 145 150 155 160

Gly Val Leu Ala Cys Tyr Ser Leu Leu Tyr Thr Val Ala Phe Ile Ile
 165 170 175

Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met
 180 185 190

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 195 200 205

Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser
 210 215 220

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Asn Arg Thr Ala Thr Leu Val Cys Asn Tyr Thr Tyr Asn Gly Thr Gly
35 40 45

Lys Glu Phe Arg Ala Ser Leu His Lys Gly Thr Asp Ser Ala Val Glu
50 55 60

Val Cys Phe Ile Ser Trp Asn Met Thr Lys Ile Asn Ser Asn Ser Asn
65 70 75 80

Lys Glu Phe Asn Cys Arg Gly Ile His Asp Lys Asp Lys Val Ile Phe
85 90 95

Asn Leu Trp Asn Met Ser Ala Ser Gln Thr Asp Ile Tyr Phe Cys Lys
100 105 110

Ile Glu Ala Met Tyr Pro Pro Pro Tyr Val Tyr Asn Glu Lys Ser Asn
115 120 125

Gly Thr Val Ile His Tyr Arg Glu Thr Pro Ile Gln Thr Gln Glu Pro
130 135 140

Glu Ser Ala Thr Ser Tyr Trp Val Met Tyr Ala Val Thr Gly Leu Leu
145 150 155 160

Gly Phe Tyr Ser Met Leu Ile Thr Ala Val Phe Ile Ile Tyr Arg Gln
165 170 175

Lys Ser Lys Arg Asn Arg Tyr Arg Gln Ser Asp Tyr Met Asn Met Thr
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 ccaaaaccca aggacaccct catgatctcc cggacccttg aggtcacatg cgtggtggtg 600
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20 25 30

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35 40 45

Tyr Ala Ser Pro Gly Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg
50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Leu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
 260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
 275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
 340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375 380

<210> 11
 <211> 1152
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EIg

<400> 11
 atgggtgtac tgctcacaca gaggacgctg ctcagtctgg tccttgcaact cctgtttcca 60
 agcatggcga gcatggcaat gcacgtggcc cagcctgctg tgggtactggc cagcagccga 120
 ggcacgcta gctttgtgtg tgagtatgca tctccaggca aagccactga ggtccgggtg 180
 acagtgcctc ggcaggctga cagccagggtg actgaagtct gtgcggcaac ctacatgatg 240

gggaatgagt tgaccttctt agatgattcc atctgcacgg gcacctccag tggaaatcaa 300
 gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg 360
 gagctcatgt acccacggcc atactacgag ggcataaggca acggaaccca gatttatgta 420
 attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480
 acatccccac cgtccccagc acctgaactc ctgggggggat cgtcagtctt cctcttcccc 540
 ccaaaaceca aggacacct catgatctcc cggacctctg aggtcacatg cgtgggtggtg 600
 gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg 660
 cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 720
 gtctcaccg tctgcacca ggactggctg aatggcaagg agtacaagt caaggtctcc 780
 aacaaagccc tccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840
 gaaccacagg tgtacacct gccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900
 ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960
 gggcagccgg agaacaacta caagaccacg cctcccgctc tggactccga cggctccttc 1020
 ttctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080
 tgctccgtga tgcattgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140
 ccgggtaaat ga 1152

<210> 12
 <211> 383
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EIg

<400> 12

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
 1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
 20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
 35 40 45

Tyr Ala Ser Pro Gly Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg

50

55

60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
 340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375 380

<210> 13

<211> 1152

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: L104EA29YIg

<400> 13

atgggtgtac tgctcacaca gaggacgctg ctcagtctgg tccttgcaact cctgtttcca	60
agcatggcga gcatggcaat gcacgtggcc cagcctgctg tggactggc cagcagccga	120
ggcatcgcta gctttgtgtg tgagtatgca tctccaggca aatatactga ggtccgggtg	180
acagtgcttc ggcaggctga cagccagggtg actgaagtct gtgcggcaac ctacatgatg	240
gggaatgagt tgaccttctt agatgattcc atctgcacgg gcacctccag tggaaatcaa	300
gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg	360
gagctcatgt acccaccgcc atactacgag ggcataggca acggaaccca gatttatgta	420
attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac	480
acatccccac cgtccccagc acctgaactc ctgggggggat cgtcagtctt cctcttcccc	540
ccaaaaccca aggacacctt catgatctcc cggacccttg aggtcacatg cgtgggtggtg	600
gacgtgagcc acgaagaccc tgagggtcaag ttcaactggt acgtggacgg cgtggaggtg	660

cataatgcc aagacaaagcc gggggaggag cagtacaaca gcaagttaccg tgtgggtcagc 720
 gtctctaccg tcttgaccca ggactggctg aatggcaagg agtacaagtg caaggtctcc 780
 aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840
 gaaccacagg tgtacaccct gcccccatcc cgggatgagc tgaccaagaa ccagggtcagc 900
 ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960
 gggcagccgg agaacaacta caagaccacg cctcccgctg tggactccga cggctccttc 1020
 ttctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080
 tgctccgtga tgcattgagc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140
 ccgggtaaat ga 1152

<210> 14
 <211> 383
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EA29YIg

<400> 14

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
 1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
 20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
 35 40 45

Tyr Ala Ser Pro Gly Lys Tyr Thr Glu Val Arg Val Thr Val Leu Arg
 50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
 65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
 85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
 100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg

340

345

350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375 380

<210> 15
 <211> 1152
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EA29LIg

<400> 15
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 agcatggcga gcatggcaat gcacgtggcc cagcctgctg tggactggc cagcagccga 120
 ggcacgcta gctttgtgtg tgagtatgca tctccaggca aattgactga ggtccgggtg 180
 acagtgcctc ggcaggctga cagccagggtg actgaagtct gtgcggcaac ctacatgatg 240
 gggaatgagt tgaccttctt agatgattcc atctgcacgg gcacctccag tggaaatcaa 300
 gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg 360
 gagctcatgt acccaccgcc atactacgag ggcataggca acggaacca gatttatgta 420
 attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480
 acatccccac cgtccccagc acctgaactc ctggggggat cgtcagtctt cctcttcccc 540
 ccaaaaccca aggacacct catgatctcc cggaccttg aggtcacatg cgtgggtggtg 600
 gacgtgagcc acgaagacc tgagggtcaag ttcaactggt acgtggacgg cgtggaggtg 660
 cataatgcc aagacaaagg gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 720
 gtctcacgg tcttgacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 780
 aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840
 gaaccacagg tgtacacct gccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900
 ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960
 gggcagccgg agaacaacta caagaccag cctcccgtgc tggactccga cggctccttc 1020
 ttctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080
 tgctccgtga tgcattgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140

ccgggtaaata ga

1152

<210> 16

<211> 383

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: L104EA29LIg

<400> 16

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
35 40 45

Tyr Ala Ser Pro Gly Lys Leu Thr Glu Val Arg Val Thr Val Leu Arg
50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
370 375 380

<210> 17

<211> 1152

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: L104EA29TIg

<400> 17

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atgggtgtac tgctcacaca gaggacgctg ctcagtctgg tccttgcaact cctgtttcca 60
agcatggcga gcatggcaat gcacgtggcc cagcctgctg tggactggc cagcagccga 120
ggcatcgcta gctttgtgtg tgagtatgca tctccaggca aaactactga ggtccgggtg 180
acagtgtctc ggcaggctga cagccagggtg actgaagtct gtgcggcaac ctacatgatg 240
gggaatgagt tgaccttctt agatgattcc atctgcacgg gcacctccag tggaaatcaa 300
gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg 360
gagctcatgt acccaccgcc atactacgag ggcataggca acggaacca gatttatgta 420
attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480
acatccccac cgtccccagc acctgaactc ctggggggat cgtcagtctt cctcttcccc 540
ccaaaacca aggacacct catgatctcc cggaccctg aggtcacatg cgtgggtggtg 600
gacgtgagcc acgaagacc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg 660
cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 720
gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 780
aacaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840
gaaccacagg tgtacacct gccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900
ctgacctgcc tggtaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960
gggcagccgg agaacaacta caagaccag cctcccgtgc tggactccga cggtccttc 1020
ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080
tgctccgtga tgcattgggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140
ccgggtaaat ga 1152
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<210> 18

<211> 383

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: L104EA29TIg

<400> 18

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala

1 5 10 15
 Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
 20 25 30
 Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
 35 40 45
 Tyr Ala Ser Pro Gly Lys Thr Thr Glu Val Arg Val Thr Val Leu Arg
 50 55 60
 Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
 65 70 75 80
 Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
 85 90 95
 Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
 100 105 110
 Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
 115 120 125
 Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
 130 135 140
 Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
 145 150 155 160
 Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
 165 170 175
 Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 180 185 190
 Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 195 200 205
 Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
 210 215 220
 Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
370 375 380

<210> 19
<211> 1152
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EA29Wig

<400> 19
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agcatggcga gcatggcaat gcacgtggcc cagcctgctg tggactggc cagcagccga 120
ggcatcgcta gctttgtgtg tgagtatgca tctccaggca aatggactga ggtccgggtg 180
acagtgttcc ggcaggctga cagccagggtg actgaagtct gtgcggcaac ctacatgatg 240
gggaatgagt tgaccttct agatgattcc atctgcacgg gcacctccag tggaaatcaa 300

gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg 360
 gagctcatgt acccaccgcc atactacgag ggcataggca acggaacca gatttatgta 420
 attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480
 acatccccac cgtccccagc acctgaactc ctggggggat cgtcagtctt cctcttcccc 540
 ccaaaaccca aggacaccct catgatctcc cggaccctcg aggtcacatg cgtgggtggtg 600
 gacgtgagcc acgaagaccc tgaggtaag ttcaactggt acgtggacgg cgtggaggtg 660
 cataatgcca agacaaagcc gcgggaggag cagtacaaca gcaegtaccg tgtgggtcagc 720
 gtcttcaccg tcttgacca ggactggctg aatggcaagg agtacaagtg caaggctctcc 780
 aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840
 gaaccacagg tgtacaccc tccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900
 ctgacctgcc tggtaaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960
 gggcagccgg agaacaacta caagaccacg cctcccgctg tggactccga cggctccttc 1020
 ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080
 tgctccgtga tgcattgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140
 ccgggtaaat ga 1152

<210> 20
 <211> 383
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EA29W1g

<400> 20

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
 1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
 20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
 35 40 45

Tyr Ala Ser Pro Gly Lys Trp Thr Glu Val Arg Val Thr Val Leu Arg
 50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu

290

295

300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
 340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375 380

<210> 21
 <211> 65
 <212> DNA
 <213> Homo sapiens

<400> 21
 ctcagtctgg tccttgcaact cctgtttcca agcatggcga gcatggcaat gcacgtggcc 60
 cagcc 65

<210> 22
 <211> 33
 <212> DNA
 <213> Homo sapiens

<400> 22
 tttgggctcc tgatcagaat ctgggcacgg ttg 33

<210> 23
 <211> 72
 <212> DNA
 <213> Homo sapiens

<400> 23
 ctagccactg aagcttcacc aatgggtgta ctgctcacac agaggacgct gctcagtctg 60
 gtccttgcaac tc 72

<210> 24
 <211> 33
 <212> DNA

<213> Homo sapiens

<400> 24

gcaatgcacg tggcccagcc tgcctgtgga gtg

33

<210> 25

<211> 45

<212> DNA

<213> Homo sapiens

<400> 25

tgaatgaaca tgcctagatc aattgatggg aataaaataa ggctg

45

<210> 26

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Oncostatin M signal peptide forward primer

<400> 26

ctagccactg aagcttcacc atgggtgtac tgctcacac

39

<210> 27

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Oncostatin M signal peptide reverse primer

<400> 27

tggcatgggc tcctgatcag gcttagaagg tccgggaaa

39

<210> 28

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Oncostatin M signal peptide reverse primer

<400> 28

tttgggctcc tgatcaggaa aatgctcttg cttggttgt

39

<210> 29

<211> 84

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Human IgGgamma1 forward prime
r

<400> 29
aagcaagagc attttcctga tcaggagccc aaatcttctg acaaaactca cacatcccca 60
ccgtccccag cacctgaact cctg 84

<210> 30

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Human IgGgamma1 reverse prime
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<400> 30
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley et al.
Serial No : 09/609,915
Filed : July 3, 2000
For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES
THEREOF

35 No. Arroyo Parkway
Pasadena, California 91103
April 12, 2002

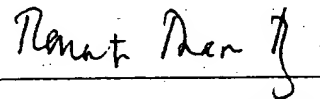
Assistant Commissioner for Patents
Box Sequence
Washington, D.C. 20231

SIR:

DECLARATION PURSUANT TO 37 C.F.R. §1.821(f)

I hereby declare that the content of the paper and computer readable copies of the Sequence Listings, submitted in the subject patent application in accordance with 37 C.F.R. §1.821(c) and (e), respectively, are the same.

Respectfully submitted,



Renato Marco P. Domingo

30436 30US12

Receipt is hereby acknowledged for the following in the U.S. Patent and Trademark Office:

Applicants: Peter S. Linsley et al.

Serial No: 09/609,915

Filed: July 3, 2000

Title: SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

Docket: 30436.30US12

Date of Deposit: May 9, 2002

RECEIVED

MAY 09 2002

Transmittal sheet, in duplicate, containing Certificate under 37 CFR §1.8

Communication in Response to Request for Substitute Papers

Exhibit 1 - Copy of Request for Substitute Papers

Exhibit 2 - Replacement computer readable form and substitute paper copy of Sequence Listing

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TECH CENTER 1600/2900

Patent

SBA

Mandel & Adriano

26-395-0694

May 8 21

12:25

P.02

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MAY 09 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

TECH CENTER 1600/2900

Applicant: Peter B. Linsley et al.

Serial No.: 09/609,915

Filed: July 3, 2000

Docket: 30436.30US12

Title: SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

CERTIFICATE UNDER 37 CFR §1.8

I hereby certify that this paper or fee is being hand delivered to Examiner Z. M. Lazar-Wesley, Ph.D. in Group 1646 in the United States Patent and Trademark Office, Washington, D.C. 20231 on May 9, 2002.

By:
Name:

STEPHANIE CUCCHERINI

35 N. Arroyo Parkway, Suite 60
Pasadena, California 91103
May 9, 2002

BOX DUPLICATE OIPR
Assistant Commissioner for Patents
P.O. Box 2327
Arlington, VA 22202-2327

Madam:

We are transmitting herewith the attached:

- ☒ Transmittal sheet, in duplicate, containing Certificate under 37 CFR §1.8
- ☒ Communication in Response to Response to Request for Substitute Papers
- ☒ Exhibit 1 - Copy of Request for Substitute Papers
- ☒ Exhibit 2 - Replacement computer readable form and Substitute paper copy of Sequence Listing
- ☒ Return postcard

Please charge any additional fees or credit overpayment to Deposit Account No. 50-0306. A duplicate of this sheet is enclosed.

MANDEL & ADRIANO

35 No. Arroyo Parkway, Suite 60
Pasadena, California 91103
(626) 395-7801

By:

Name: Sara Lynn Mandel

Reg. No.: 31,853

Initials: SLM

Customer No. 26,941

RECEIVED

RECEIVED

MAY 09 2002

TECH CENTER 1600/2900

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Peter S. Linsley et al.
Serial No.: 09/609,915
Filed: July 3, 2000
Docket: 30436.30US12
Title: SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

CERTIFICATE UNDER 37 CFR §1.8

I hereby certify that this paper or fee is being hand delivered to Examiner E. M. Lazar-Wesley, Ph.D. in Group 1646 in the United States Patent and Trademark Office, Washington, D.C. 20231 on May 9, 2002.

By: 

Name:

STEPHANIE CUCCCHERINI

35 N. Arroyo Parkway, Suite 60
Pasadena, California 91103
May 9, 2002

BOX DUPLICATE OIPE
Assistant Commissioner for Patents
P.O. Box 2327
Arlington, VA 22202-2327

Madam:

We are transmitting herewith the attached:

- ☒ Transmittal sheet, in duplicate, containing Certificate under 37 CFR §1.8
- ☒ Communication in Response to Response to Request for Substitute Papers
- ☒ Exhibit 1 - Copy of Request for Substitute Papers
- ☒ Exhibit 2 - Replacement computer readable form and Substitute paper copy of Sequence Listing
- ☒ Return postcard

Please charge any additional fees or credit overpayment to Deposit Account No. 50-0306. A duplicate of this sheet is enclosed.

MANDEL & ADRIANO

35 No. Arroyo Parkway, Suite 60
Pasadena, California 91103
(626) 395-7801

By: 

Name: SaraLynn Mandel
Reg. No.: 31,853
Initials: SLM
Customer No. 26,941

Dkt. 30436.30USI2/SBA/HP

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley, et al.

Serial No. : 09/609,915

Examiner: E. M. Lazar-Wesley, Ph.D.

Filed : July 3, 2000

Group Art Unit: 1646

For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES
THEREOF35 North Arroyo Pkwy, Suite 60
Pasadena, California 91103
May 8, 2002Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

**COMMUNICATION IN RESPONSE TO
REQUEST FOR SUBSTITUTE PAPERS**

The U.S. Patent Office issued a Request for Substitute Papers, dated April 29, 2002, in connection with the above-referenced patent application. A Response to the Request is due May 13, 2002. Accordingly, this Response is being timely filed. A copy of the Request is submitted hercin as Exhibit 1.

In the Request, the Patent Office states that the papers filed on 04/27/02 with CD are no longer in condition to become part of the permanent records of the United States Patent and Trademark Office (USPTO) for this application (37 CFR 1.52 (a)) due to the United States Postal Service sanitization process.

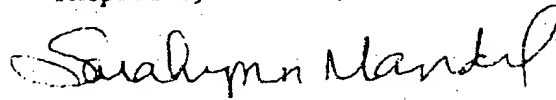
Applicants provide herein a copy of the above-identified papers including a Preliminary Amendment, Replacement computer readable form and Substitute paper copy of Sequence listing (Exhibit 2). The enclosed papers are a true and accurate copy of the above-identified papers, which were filed with the USPTO on April 15, 2002. The

Applicants: Peter S. Linsley et al.
U.S. Serial No. 09/609,913
Filed: July 3, 2000
Page 2

Replacement computer readable form and Substitute paper copy contain no new matter and their entry is respectfully requested.

No fee is deemed necessary in connection with the filing of this Response. However, if any fee is necessary, the Patent Office is authorized to charge any additional fee to Deposit Account No. 50-0306.

Respectfully submitted,



Sarah B. Adriano
Registration No. 34,470
SaraLynn Mandel
Registration No. 31,853
Patent Practitioners for Applicants
Mandel & Adriano
35 N. Arroyo Parkway
Pasadena, California 91103
(626) 395-7801
Customer No. 26,941

EXHIBIT 1

Ser. No. 09/609,915

**Copy of the
Request for Substitute Papers**



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20591
www.uspto.gov

Application Number	Filing Date	First Named Applicant	Atty. Docket No.
097609,913	07/03/2000	Linsley, Peter	30436.30UST1

Mandel & Adriano
35 N Arroyo Parkway
Suite 60
Pasadena CA 91103

Title: Soluble CTLA4 mutant molecules and uses thereof

Date Mailed: 04/29/2002

Request for Substitute Papers

The papers filed on 04/22/02 with CDs are no longer in condition to become part of the permanent records of the United States Patent and Trademark Office (USPTO) for this application (37 CFR 1.52(a)) due to the United States Postal Service sanitization process.

The USPTO requests that applicant provide a copy of the above-identified papers (except for any U.S. or foreign patent documents submitted with the above-identified papers) with a statement that such copy is a complete and accurate copy of the above-identified papers (signing and returning a copy of this notice will provide such a statement). The reply to this letter should be submitted to the USPTO by facsimile at the number indicated ~~703-746-9195~~ 703-746-9195

Alternatively, the reply to this letter may be hand-carried to the Customer Service Window located in Room 1B03 of Crystal Plaza Building 2, Arlington, Virginia, 22202.

The USPTO strongly prefers that the reply to this letter be submitted by facsimile. However, if applicant cannot submit the reply to this letter by facsimile (or hand-delivery), the reply may be mailed to: Box Duplicate OIPE, U.S. Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202-2327.

This letter is not a notice under 37 CFR 1.251. However, failure to timely reply to this notice within two (2) weeks of the date of receipt of this letter may result in the USPTO issuing a notice under 37 CFR 1.251. A copy of this notice should be included with the reply.

The enclosed papers are a complete and accurate copy of the above-identified papers.

Name: SARALYN MANDU Registration No.: 31,853

Signature: Saralyn Mandu Date: 5/9/02

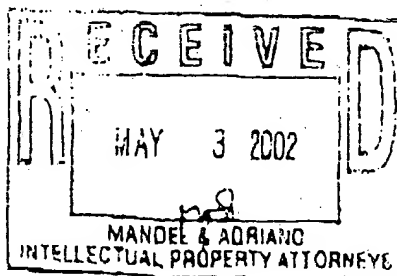


EXHIBIT 2

Ser. No. 09/609,915

**Copy of the Papers Filed on April 15,
2002, including a Preliminary
Amendment, Replacement computer
readable form and Substitute paper
copy of Sequence listing**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Peter S. Linsley et al.

Serial No.: 09/609,915

Examiner: E. M. Lazar-Wesley, Ph.D.

Filed: July 3, 2000

Group Art Unit: 1646

For: SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

35 N. Arroyo Pkwy., Suite 60
Pasadena, California 91103
April 15, 2002

Honorable Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

**COMMUNICATION IN RESPONSE TO A NOTICE TO COMPLY WITH
REQUIREMENTS OF 37 CFR 1.821-1.825 FOR PATENT APPLICATIONS
CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE
DISCLOSURES DATED MARCH 15, 2002**

This communication is submitted in response to an Office Communication dated March 15, 2002, to comply with requirements of 37 C.F.R. 1.821 through 1.825 for sequence listing, in connection with the above-identified application. A one (1) month period for reply was set, making April 15, 2002, the deadline for filing a response to the Notice. Accordingly, this Response is being timely filed. A copy of the above-referenced Notice is submitted herein as Exhibit 1.

In response to the Office Communication, the applicants herein submit a sequence listing including an original paper copy, a computer readable copy, and a Declaration under 37 C.F.R. §1.821(f) stating that the computer readable copy of the sequence listing is identical to the paper

Applicants: Peter S. Lins et al.
U.S. Serial No. 09/609,915
Filed: July 3, 2000
Page 2

copy (Exhibit 2). The sequence listing contains no new matter and is supported by the specification as originally filed. Accordingly, entry of this amendment is respectfully requested.

No additional fee is deemed necessary in connection with the filing of this Communication. However, if any additional fee is necessary, the Patent Office is authorized to charge the additional fee to Deposit Account No. 50-0306.

Respectfully submitted,

Sarah B. Adriano

Sarah B. Adriano
Registration No. 34,470
SaraLynn Mandel
Registration No. 31,853
Attorneys for Applicants
Mandel & Adriano
35 N. Arroyo Parkway
Pasadena, California 91103
(626) 395-7801
Customer Number: 26941

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley, et al.
Serial No. : 09/609,915 Examiner: E. M. Lazar-Wesley, Ph.D.
Filed : July 3, 2000 Group Art Unit: 1646
For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES
THEREOF

35 North Arroyo Pkwy, Suite 60
Pasadena, California 91103
April 15, 2002

Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

PRELIMINARY AMENDMENT

Please amend the subject application as follows.

In the Specification:

In accordance with 37 C.F.R. 1.121(a)(1)(i) and (ii), please replace the paragraph at page 1, lines 5-10, with the following rewritten paragraph:

-- This application is a continuation-in-part of U.S. Serial No. 08/228,208, filed April 15, 1994, now U.S. Patent No. 6,090,914, issued July 18, 2000, which was a continuation-in-part of U.S. Serial No. 08/008, 898, filed January 22, 1993, now U.S. Patent No. 5,770,197, issued June 23, 1998, which was a continuation-in part of U.S. Serial No. 07/723,617, filed June 27, 1991, now abandoned; U.S. Serial No. 09/603,825, filed June 26, 2000, which was a continuation-in-part of U.S. Serial No. 09/014,761, filed January 28, 1998, which claims priority of U.S. Serial No. 60/036,594, filed January 31, 1997, now abandoned; and U.S. Serial No. 08/539,436, filed October 5, 1995, now U.S. Patent No. 6,132,992, issued

October 17, 2000, the contents of all of which are hereby incorporated by reference, in their entirety, into the present application. --.

Please replace the paragraph at page 4, lines 14-16, beginning "Figure 3:", with the following rewritten paragraph:

-- Figure 3: Shows the nucleotide (SEQ ID NO.: 1) and complete amino acid sequence (SEQ ID NO.: 2) encoding human CTLA4 receptor fused to the oncostatin M signal peptide, and including the newly identified N-linked glycosylation site, as described in Example 1, infra. --.

Please replace the paragraph at page 6, lines 20-25, please replace the paragraph beginning "Figure 7:", with the following rewritten paragraph:

-- Figure 17: Depicts sequencing alignment of CD28 and CTLA4 family members. Sequences of human (H) (SEQ ID NO.: 7), mouse (M) (SEQ ID NO.: 5), rat (R) (SEQ ID NO.: 6), and chicken (Ch) CD28 (SEQ ID NO.: 8) are aligned with human (SEQ ID NO.: 3) and mouse CTLA4 (SEQ ID NO.: 4). The signal peptides are underlined with a dashed line. The transmembrane domains are underlined with a solid line. The CDR-analogous regions are noted. The dark shaded areas highlight complete conservation of residues while the light shaded areas highlight conservative amino acid substitutions in all family members.--.

Please replace the paragraph at page 7, lines 12-13, beginning "Figure 22:", with the following rewritten paragraph:

-- Figure 22: Depicts the nucleotide (SEQ ID NO.: 9) and amino acid sequence of a CTLA4Ig (SEQ ID NO.: 10) having wildtype extracellular domain of CTLA4. --.

Please replace the paragraph at page 7, lines 15-17, beginning "Figure 23:", with the following rewritten paragraph:

-- Figure 23: Depicts the nucleotide (SEQ ID NO.: 11) and amino acid (SEQ ID NO.: 12) sequences of L104EIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.--.

Please replace the paragraph at page 7, lines 19-21, beginning "Figure 24:", with the following rewritten paragraph:

-- Figure 24: Depicts the nucleotide (SEQ ID NO.: 13) and amino acid sequence (SEQ ID NO.: 14) of L104EA29YIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.--

Please replace the paragraph at page 7, lines 23-25, beginning "Figure 25:", with the following rewritten paragraph:

-- Figure 25: Depicts the nucleotide (SEQ ID NO.: 15) and amino acid sequences (SEQ ID NO.: 16) of L104EA29LIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124. --

Please replace the paragraph at page 7, lines 27-29, beginning "Figure 26:", with the following rewritten paragraph:

-- Figure 26: Depicts the nucleotide (SEQ ID NO.: 17) and amino acid sequences (SEQ ID NO.: 18) of L104EA29TIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124. --

Please replace the paragraph at page 8, lines 1-3, beginning "Figure 27:", with the following rewritten paragraph:

-- Figure 27: Depicts the nucleotide (SEQ ID NO.: 19) and amino acid sequences (SEQ ID NO.: 20) of L104EA29WIIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.--

Please replace the paragraph at page 9, lines 22-25, beginning "Figure 37:", with the following rewritten paragraph:

-- Figure 37: Depicts the results of a FACS assay, showing L104EIg and L104ES25RIg bind CHO cells stably transfected with human CD80 or CD86. A) L104EIg and

L104ES25RIg bind to human CD80 CHO-transfected cells; B) L104EIg and L104ES25RIg bind to human CD86 CHO-transfected cells.---

Please replace the paragraph at page 11 lines 1-8, beginning "One embodiment", with the following rewritten paragraph:

-- One embodiment of a soluble CTLA4 has been deposited with the American Type Culture Collection (ATCC) in Manassas, Maryland, under the provisions of the Budapest Treaty on May 31, 1991 and has been accorded ATCC accession number: 68629. ATCC 68629 is DNA encoding *CTLA4*Ig. Additionally, the CTLA4Ig-24 CHO cell line has been deposited with the ATCC under the Budapest Treaty on May 31, 1991 and has been accorded accession number ATCC 10762. DNA encoding L104EA29YIg was submitted for deposit on June 19, 2000 with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209. The DNA encoding L104EA29YIg has been accorded ATCC accession number PTA-2104. --

Please replace the paragraph at page 33, lines 2-21, beginning "Because a signal peptide", with the following rewritten paragraph:

-- Because a signal peptide for CTLA4 was not identified in the CTLA4 gene, the N-terminus of the predicted sequence of CTLA4 was fused to the signal peptide of oncostatin M (Malik et al., Mol. and Cell. Biol. 9:2847 (1989)) in two steps using overlapping oligonucleotides.

For the first step, the oligonucleotide,

CTCAGTCTGGTCCTTGCACTCCTG

TTTCCAAGCATGGCGAGCATGGCAATGCACGTGGCCCAGCC (SEQ ID NO.: 21)

(which encoded the C terminal 15 amino acids from the oncostatin M signal peptide fused to the N terminal 7 amino acids of CTLA4) was used as forward primer, and TTTGGGCTCCTGATCAGAATCTGGGCACGGTTG (SEQ ID NO.: 22) (encoding amino acid residues 119-125 of the amino acid sequence encoding CTLA4 receptor and containing a *Bel I* restriction enzyme site) as reverse primer. The template for this step was cDNA synthesized from 1 micro g of total RNA from H38 cells (an HTLV II infected T cell leukemic cell line provided by Drs. Salahudin and Gallo, NCI, Bethesda, MD). A portion of

the PCR product from the first step was reamplified, using an overlapping forward primer, encoding the N terminal portion of the oncostatin M signal peptide and containing a Hind III restriction endonuclease site,

CTAGCCACTGAAGCTTCACCAATGGGTGTACTGCTCACACAGAGGACGCTGCT
CAGTCTGGTCCTTGCACTC (SEQ ID NO.: 23) and the same reverse primer. The product of the PCR reaction was digested with Hind III and Bcl I and ligated together with a Bcl I/Xba I cleaved cDNA fragment encoding the amino acid sequences corresponding to the hinge, CH2 and CH3 regions of IgC γ 1 into the Hind III/Xba I cleaved expression vector, CDM8 or Hind III/Xba I cleaved expression vector pLN. --.

Please replace the paragraph at page 33, lines 23-29, beginning "A schematic map", with the following rewritten paragraph:

-- A schematic map of the resulting CTLA4Ig fusion construct is shown in Figure 1. Sequences displayed in this figure show the junctions between CTLA4 (upper case letters, unshaded regions) and the signal peptide, SP, of oncostatin M (dark shaded regions), and the hinge, H, of IgC(gamma)1 (stippled regions). The amino acid in parentheses was introduced during construction. Asterisks (*) indicate cysteine to serine mutations introduced in the IgC γ hinge region. The immunoglobulin superfamily V-like domain present in CTLA4 is indicated, as are the CH2 and CH3 domains of IgC(gamma)1. --.

Please replace the paragraph at page 34, lines 21-27, beginning "CTLA4Ig", with the following rewritten paragraph:

-- CTLA4Ig was purified by protein A chromatography from serum-free conditioned supernatants (Figure 2). Concentrations of CTLA4Ig were determined assuming an extinction coefficient at 280 nm of 1.6 (experimentally determined by amino acid analysis of a solution of known absorbance). Molecular weight standards (lanes 1 and 3, Figure 2) and samples (1 micro grams) of CTLA4Ig (lanes 2 and 4) were subjected to SDS-PAGE (4-12% acrylamide gradient) under non-reducing conditions (- beta ME, lanes 1 and 2) or

reducing conditions (+ beta ME, lanes 3 and 4) Proteins were visualized by staining with Coomassie Brilliant Blue. --.

Please replace the paragraph at page 35, lines 19-31, beginning "Because of expression of CTLA4 receptor", with the following rewritten paragraph:

-- Because the expression of CTLA4 receptor protein in human lymphoid cells has not been previously reported, it was necessary to locate a source of CTLA4 mRNA. cDNA was reverse transcribed from the total cellular RNA of H38 cells, as noted above, was used for cloning by PCR. For this purpose, the oligonucleotide, GCAATGCACGTGGCCCAGCCTGCTGTGGTAGTG (SEQ ID NO.: 24) (encoding the first 11 amino acids in the predicted coding sequence) was used as a forward primer, and TGATGTAACATGTCTAGATCAATTGATGGGAATAAAATAAGGCTG (SEQ ID NO.: 25) (homologous to the last 8 amino acids in CTLA4 and containing a Xba I site) as reverse primer. The template again was a cDNA synthesized from 1 micro gram RNA from H38 cells. Products of the PCR reaction were cleaved with the restriction endonucleases Nco I and Xba I and the resulting 316 bp product was gel purified. A 340 bp Hind III/Nco I fragment from the CTLA4Ig fusion described above was also gel-purified, and both restriction fragments were ligated into Hind III/Xba I cleaved CDM8 to form OMCTLA. --.

Please replace the paragraph at page 36, lines 21-25, beginning "Receptor-immunoglobulin C gamma", with the following rewritten paragraph:

-- Receptor-immunoglobulin C gamma (IgC γ) fusion proteins B7Ig and CD28Ig were prepared as described by Linsley et al., in J. Exp. Med. 173:721-730 (1991), incorporated by reference herein. Briefly, DNA encoding amino acid sequences corresponding to the respective receptor protein (e.g. B7) was joined to DNA encoding amino acid sequences corresponding to the hinge, CH2 and CH3 regions of human IgC γ 1. This was accomplished as follows. --.

Please replace the paragraph at page 37, lines 6-25, beginning "Plasmid Construction", with the following rewritten paragraph:

-- Plasmid Construction. Expression plasmids containing cDNA encoding CD28, (as described by Aruffo and Seed, Proc. Natl. Acad. Sci. USA 84:8573 (1987)), were provided by Drs. Aruffo and Seed (Mass General Hospital, Boston, MA). Plasmids containing cDNA encoding CD5, (as described by Aruffo, Cell 61:1303 (1990)), were provided by Dr. Aruffo. Plasmids containing cDNA encoding B7, (as described by Freeman et al., J. Immunol. 143:2714 (1989)), were provided by Dr. Freeman (Dana Farber Cancer Institute, Boston, MA). For initial attempts at expression of soluble forms of CD28 and B7, constructs were made (OMCD28 and OMB7) as described by Linsley et al., J. Exp. Med., *supra*, in which stop codons were introduced upstream of the transmembrane domains and the native signal peptides were replaced with the signal peptide from oncostatin M (Malik et al., Mol. Cell Biol. 9:2847 (1989)). These were made using synthetic oligonucleotides for reconstruction (OMCD28) or as primers (OMB7) for PCR. OMCD28, is a CD28 cDNA modified for more efficient expression by replacing the signal peptide with the analogous region from oncostatin M. CD28Ig and B7Ig fusion constructs were made in two parts. The 5' portions were made using OMCD28 and OMB7 as templates and the oligonucleotide, CTAGCCACTGAAGCTTCACCATGGGTGTACTGCTCACAC (SEQ ID NO.: 26) (encoding the amino acid sequence corresponding to the oncostatin M signal peptide) as a forward primer, and either TGGCATGGGCTCCTGATCAGGCTTAGAAGGTCCGGGAAA (SEQ ID NO.: 27) or, TTTGGGCTCCTGATCAGGAAAATGCTCTTGCTTGGTTGT (SEQ ID NO.: 28) as reverse primers, respectively. Products of the PCR reactions were cleaved with restriction endonucleases (Hind III and BclI) as sites introduced in the PCR primers and gel purified.--

Please replace the paragraph at page 37, line 27, through page 38, line 9, beginning "The 3' portion of the fusion constructs", with the following rewritten paragraph:

-- The 3' portion of the fusion constructs corresponding to human IgC γ 1 sequences was made by a coupled reverse transcriptase (from Avian myeloblastosis virus; Life Sciences

Associates, Bayport, NY)-PCR reaction using RNA from a myeloma cell line producing human-mouse chimeric mAb L6 (provided by Dr. P. Fell and M. Gayle, Bristol-Myers Squibb Company, Pharmaceutical Research Institute, Seattle, WA) as template. The oligonucleotide,

AAGCAAGAGCATTTCCTGATCAGGAGCCCAAATCTTCTGACAAACTCACA
CATCCCCACCGTCCCCAGCACCTGAACTCCTG (SEQ ID NO.: 29) was used as forward primer,

CTTCGACCAGTCTAGAAGCATCCTCGTGCGACCGCGAGAGC (SEQ ID NO.: 30) as reverse primer. Reaction products were cleaved with BclI and XbaI and gel purified. Final constructs were assembled by ligating HindIII/BclI cleaved fragments containing CD28 or B7 sequences together with BclI/XbaI cleaved fragment containing IgC γ 1 sequences into HindIII/XbaI cleaved CDM8. Ligation products were transformed into MC1061/p3 *E. coli* cells and colonies were screened for the appropriate plasmids. Sequences of the resulting constructs were confirmed by DNA sequencing. --

Please replace the paragraph at page 38, lines 17-24, beginning "CD5Ig was constructed in identical fashion, using", with the following rewritten paragraph:

-- CD5Ig was constructed in identical fashion, using
CATTGCACAGTCAAGCTTCCATGCCCATGGGTTCTCTGGCCACCTTG (SEQ ID
NO.: 31) as forward primer and
ATCCACAGTGCAGTGATCATTGGATCCTGGCATGTGAC (SEQ ID NO.: 32) as
reverse primer. The PCR product was restriction endonuclease digested and ligated with the
IgC γ 1 fragment as described above. The resulting construct (CD5Ig) encoded a mature
protein having an amino acid sequence containing amino acid residues from position 1 to
position 347 of the sequence corresponding to CD5, two amino acids introduced by the
construction procedure (amino acids DQ), followed by DNA encoding amino acids
corresponding to the IgC γ 1 hinge region. --

Please replace the paragraph at page 39, lines 21-30, beginning "Immunostaining and FACS^R Analysis," with the following rewritten paragraph:

-- Immunostaining and FACS^R Analysis: Transfected CHO or COS cells or activated T cells were analyzed by indirect immunostaining. Before staining, CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with murine mAbs 9.3 (Hansen et al., Immunogenetics 10:247 (1980)) or BB-1 (Yokochi et al., J. Immunol. 128:823 (1981)), or with Ig fusion proteins (all at 10 micrograms/ml in DMEM containing 10% FCS) for 1-2 h at 4 °C. Cells were then washed, and incubated for an additional 0.5-2h at 4 °C with a FITC-conjugated second step reagent (goat anti-mouse Ig serum for murine mAbs, or goat anti-human Ig G_γ serum for fusion proteins (Tago, Inc., Burlingame, CA)). Fluorescence was analyzed on a FACS IV^R cell sorter (Becton Dickinson and CO., Mountain View, CA) equipped with a four decade logarithmic amplifier. --.

Please replace the paragraph at page 40, line 28, through page 41, line 5, beginning "mAbs," with the following rewritten paragraph:

-- mAbs. Murine monoclonal antibodies (mAbs) 9.3 (anti-CD28) and G19-4 (anti-CD3), G3-7 (anti-CD7), BB-1 (anti-B7 antigen) and rat mAb 187.1 (anti-mouse kappa chain) have been described previously (Ledbetter et al., Proc. Natl. Acad. Sci. 84:1384-1388 (1987); Ledbetter et al., Blood 75:1531 (1990); Yokochi et al., supra) and were purified from ascites before use. The hybridoma producing mAb OKT8 was obtained from the ATCC, Rockville, MD, and the mAb was also purified from ascites before use. mAb 4G9 (anti-CD19) was provided by Dr. E. Engleman, Stanford University, Palo Alto, CA). Purified human-mouse chimeric mAb L6 (having human C_γ1 Fc portion) was a gift of Dr. P. Fell and M. Gayle (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA). --.

Please replace the paragraph at page 41, lines 7-15, beginning "Immunostaining and FACS^R

Analysis," with the following rewritten paragraph:
-- Immunostaining and FACS^R Analysis. Prior to staining, COS or CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with mAbs or Ig fusion proteins at 10 micro grams/ml in DMEM containing 10% FBS for 1-2 hr at 4 degrees C. Cells were then washed, and incubated for an additional 0.5-2 hrs at 4 degrees C with FITC-conjugated goat anti-mouse immunoglobulin or with FITC-conjugated goat anti-human IgG serum (both from Tago, Burlingame, CA). When binding of both mAbs and Ig fusion proteins were measured in the same experiment, FITC-conjugated anti-mouse and anti-human second step reagents were mixed together before use. Fluorescence on a total of 10,000 cells was then analyzed by FACS^R. --.

Please replace the paragraph at page 44, lines 10-17, beginning "Binding of CTLA4Ig on B7

Positive CHO cells," with the following rewritten paragraph:

-- Binding of CTLA4Ig on B7 Positive CHO cells. To further characterize the binding of CTLA4Ig and B7, the binding activity of purified CTLA4Ig on B7⁺ CHO cells and on a lymphoblastoid cell line (PM LCL) was measured in the experiment shown in Figure 5. Amplified transfected CHO cell lines and PM LCLs were incubated with medium only (no addition) or an equivalent concentration of human IgG1-containing proteins (10 micro grams/ml) of CD5Ig, CD28Ig or CTLA4Ig. Binding was detected by FACS^R following addition of FITC-conjugated goat anti-human Ig second step reagents. A total of 10,000 stained cells were analyzed by FACS^R. --.

Please replace the paragraph at page 46, lines 14-19, beginning "Primary mixed lymphocyte reaction (MLR)", with the following rewritten paragraph:

-- Primary mixed lymphocyte reaction (MLR) blasts were stimulated with irradiated T51 lymphoblastoid cells (LC) in the absence or presence of concentrations of murine mAb 9.3 Fab fragments, or B7Ig, CD28Ig or CTLA4Ig immunoglobulin G fusion proteins. Cellular

proliferation was measured by [³H]-thymidine incorporation after 4 days and is expressed as the percentage of incorporation by untreated cultures (21,000 cpm). Figure 9 shows the mean of quadruplicate determinations (SEM \leq 10%).--.

Please replace the paragraph at page 48, lines 8-17, beginning "These results demonstrate", with the following rewritten paragraph:

-- These results demonstrate the first expression of a functional protein product of CTLA4 transcripts. CTLA4Ig, a fusion construct containing the extracellular domain of CTLA4 fused to an IgC γ 1 domain, forms a disulfide-linked dimer of M_r approximately 50,000 subunits. Because no interchain disulfides would be predicted to form in the Ig portion of this fusion, it seems likely that cysteines from CTLA4 are involved in disulfide bond formation. The analogous CD28Ig fusion protein (Linsley et al, supra, 1991) also contains interchain disulfide linkage(s). These results suggest that CTLA4 receptor, like CD28 (Hansen et al., Immunogenetics 10:247-260 (1980)), exists on the T cell surface as a disulfide linked homodimer. Although CD28 and CTLA4 are highly homologous proteins, they are immunologically distinct, because the anti-CD28 mAb, mAb 9.3, does not recognize CTLA4 (Figures 4 and 5). --

Please replace the paragraph at page 57, lines 20-23, beginning "In addition, two mutants", with the following rewritten paragraph:

-- In addition, two mutants encoding the residues P103A and Y104A (MYPPAY (SEQ ID NO.: 40) and MYPPPA (SEQ ID NO.: 41), respectively) from the CD28Ig 99MYPPPY104 hexapeptide using CD28Ig as a template were also prepared by the same method. --.

Please replace the paragraph at page 57, line 30, through page 58, line 1, beginning "These primers encoded the following sequences:", with the following rewritten paragraph:

-- These primers encoded the following sequences:

CDMSFP:5'-AATACGACTCACTATAGG (SEQ ID NO.: 33)

CDM8RP:5'-CACCACACTGTATTAACC (SEQ ID NO.: 34)

Please replace the paragraph at page 58, line 29, through page 59, line 2, beginning "HS7, HS8, and HS9 constructs", with the following rewritten paragraph:

-- HS7, HS8, and HS9 constructs were prepared by replacing a ~350 base-pair HindIII/HpaI 5' fragment of HS4, HS4-A, and HS4-B, respectively, with the equivalent cDNA fragment similarly digested from HS5 thus introducing the CDR1-like loop of CTLA4 into those hybrids already containing the CTLA4 CDR3-like region. --.

Please replace the paragraph at page 62, lines 29-31, beginning "Several versions of the model", with the following rewritten paragraph:

-- Several versions of the model with modified assignments of some residues to β -strands or loops were tested using 3D-profile analysis (Luthy et al., 1992, Nature 336:83-85) in order to improve the initial alignment of the CTLA4 extracellular region sequence with an IGSF variable fold. --.

Please replace the paragraph at page 63, lines 12-15, beginning "Regions of sequence conservation", with the following rewritten paragraph:

-- Regions of sequence conservation are scattered throughout the extracellular domains of these proteins with the most rigorous conservation seen in the hexapeptide MYPPPY (SEQ ID NO.: 35) motif located in the CDR3-like loop of both CTLA4 and CD28 (Figure 17). This suggests a probable role for this region in the interaction with a B7 antigen, e.g., B7-1 and B7-2. --.

Please replace the TABLE B at page 73, lines 1-45, beginning "TABLE B. Binding of CTLA4 and CD28 monoclonal antibodies", with the following rewritten paragraph:

-- **TABLE B.** Binding of CTLA4 and CD28 monoclonal antibodies to CTLA4Ig and CD28Ig mutant fusion proteins and to CTLA4/CD28Ig hybrid fusion proteins.

	<u>anti-CTLA4 mAbs</u>		<u>anti-CD28 mAb</u>
	7F8	11D4	10A8
			9.3
<u>CTLA4Ig MUTANT FUSION PROTEIN</u>			
AYPPPY (SEQ ID NO.: 36)	+++	+++	+++
MAPPPY (SEQ ID NO.: 37)	++	+	++
MYAPPY (SEQ ID NO.: 38)	+	-	+
MYPAPY (SEQ ID NO.: 39)	+++	+++++	+++
MYPPAY (SEQ ID NO.: 40)	+++	-	+
MYPPPA (SEQ ID NO.: 41)	+++	++	+++
AAPPPY (SEQ ID NO.: 42)	+	++	+++
<u>CD28Ig MUTANT FUSION PROTEIN</u>			
MYPPAY (SEQ ID NO.: 40)	-	-	-
MYPPPA (SEQ ID NO.: 41)	-	-	+
<u>CTLA4/CD28Ig HYBRID FUSION PROTEINS</u>			
HS1	-	-	-
HS2	-	-	+
HS3	-	-	-
HS4	-	-	+++
HS5	-	-	-
HS6	+	-	-
HS4-A	-	-	++
HS4-B	-	-	++
HS7	-	-	+++
HS8	-	+	+++
HS9	-	+	-
HS10	-	-	-
HS11	-	-	+
HS12	-	-	-
HS13	-	-	-
HS14	-	-	-
CTLA4Ig	+++	+++	+++
CD28Ig	-	-	+++

Antibody binding was rated from that seen for wild type protein (+++) to above background (+), and no detectable binding (-).

Please replace the paragraph at page 77, lines 12-17, with the following rewritten paragraph:

-- Experimental data were first fit to a model for a single ligand binding to a single receptor (1-site model, i.e., a simple langmuir system, $A+B \rightleftharpoons AB$), and equilibrium association constants ($K_d = [A] \cdot [B] / [AB]$) were calculated from the equation $R = R_{max} \cdot C / (K_d + C)$. Subsequently, data were fit to the simplest two-site model of ligand binding (i.e., to a receptor having two non-interacting independent binding sites as described by the equation $R = R_{max1} \cdot C / (K_{d1} + C) + R_{max2} \cdot C / (K_{d2} + C)$).

Please replace the paragraph at page 84, lines 23-28, beginning "From tyrosine +23 to threonine +30," with the following rewritten paragraph:

-- From tyrosine +23 to threonine +30, a pool of degenerate forward primers were generated having the following sequence:

5' CGA GGC ATC GCT AGC TTT GTG TGT GAG XXK XXK XXK XXK XXK XXK
XXK XXK GAG GTC CGG GTG ACA GT 3' (SEQ ID NO.: 43)

Where X = any of the four nucleotides A, T, G, or C and K = either T, G, or C

Each primer contained a Nhe I restriction enzyme cut site. --

Please replace the paragraph at page 84, lines 30-32, beginning "The reverse primer had the following sequence:" with the following rewritten paragraph:

-- The reverse primer had the following sequence:

5' GGT TGC CGC ACA GAC TTC GGT CAC CTG GCT GTC AGC CTG CCG AAG
CAC TGT CAC CCG GA 3' (SEQ ID NO.: 44) --

Please replace the paragraph at page 86, lines 24-31, beginning "Five mutants were enriched through these 5 rounds of panning," with the following rewritten paragraph:

-- Five mutants were enriched through these 5 rounds of panning.

Mut 9	F-E-P-K-R-G-V-Q (SEQ ID NO.: 45)
Mut 19	W-D-Q-Y-T-G-Y-G (SEQ ID NO.: 46)
Mut 71	W-D-A-Y-R-N-Q-Q (SEQ ID NO.: 47)

Mut 45 Y-D-H-P-Y-D-G-Q (SEQ ID NO.: 48)
Mut 4 W-D-Q-H-V-S-R-R (SEQ ID NO.: 49)
CTLA4 Y-A-S-P-G-K-A-T (SEQ ID NO.: 50)

REMARKS

The changes to the specification update the priority claimed in the subject application, provide SEQ ID NOs and ATCC accession number, and correct typographical errors in the subject application.

The amendments to specification at page 1, lines 5-10 merely update the status of the priority documents for the subject application. The amended priorities are supported by the executed combined Declaration and Power of Attorney submitted with the subject application. Thus, the above amendments do not introduce any new matter, and accordingly their entry is respectfully requested.

The amendments to specification at pages 4, lines 14-16; page 6, lines, 20-25; page 7, lines 12-13; page 7, lines 15-17; page 7, lines 19-21; page 7, lines, 23-25; page 7, lines 27-29; page 8, lines 1-3; page 33, lines 1-21; page 35, lines 19-31; page 37, lines 6-25; page 38, lines 1-9 and 17-24; page 57, lines 20-22 and 33; page 58, line 1; page 63, lines 12-15; page 73, lines 10-21; page 84, lines 23-28 and 30-32; page 86, lines 24-31 are merely to provide SEQ ID NOs in the Detailed Description. A sequence listing, including a paper copy, a computer readable form and a Declaration pursuant to 37 C.F.R. §1.821(f) are submitted herein as Exhibit 2. The amendments to incorporate SEQ ID NOs. do not introduce any new matter and are supported by the disclosure as originally filed. Accordingly, entry of these amendments is respectfully requested.

The amendments to specification, at page 11, lines 1-8, provides the ATCC accession number for the DNA encoding L104EA29Ylg which was deposited with the ATCC under the provision of Budapest treaty and appropriately referenced in the originally filed

application. The amendment to incorporate the ATCC accession number for the deposited DNA does not introduce any new matter, and accordingly the entry of the amendment is respectfully requested.

The amendments to specification, at page 9, lines 22-25 merely deletes "Figure 35" to correct a typographical error. The above amendment does not introduce any new matter, and accordingly the entry of the amendment is respectfully requested.

The amendments to specification at page 33, lines 19 and 27; page 36, lines 21-25; page 37, line 27; page 38, lines 6 and 17-24; page 39, line 28; page 41, lines 4 and 12; page 44, line 14; page 46, line 16; and page 48, line 10, incorporates the symbol γ to correct a typographical error for IgC γ 1. The IgC γ 1 is a commonly used abbreviation in the art for IgC gamma1. The term "IgC gamma1" is supported by the specification as originally filed (see pages 11, line 14; 27, line 29, 32, line 29). The above amendment is further supported by U.S. Serial No. 08/228,208 (see page 36, line 27, page 40, line 25), to which this application claims priority. Thus, the above amendments do not introduce any new matter, accordingly their entry is respectfully requested.

The amendments to specification at page 34, line 26, merely incorporates -- beta -- to correct a typographical error. The support for the amendment can be found on page 34, line 26 of the specification as originally filed. The above amendment does not introduce any new data, accordingly its entry is respectfully requested.

The amendment to specification at page 58, line 29, corrects a typographical error by incorporating -- ~ --. The above amendment is supported by U.S. Serial No. 08/228,208 (page 64, line 18), to which this application claims priority. The amendment to correct the typographical error at page 58, line 29 does not incorporate any new data, and accordingly, its entry is respectfully requested.

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The amendment to specification at page 62, line 29, corrects a typographical error by incorporating the symbol β for beta strands. The above amendment is supported by U.S. Serial No. 08/228,208 (page 68, line 27), to which this application claims priority. The above amendment does not introduce any new matter, and accordingly, its entry is respectfully requested.

The amendment to specification at page 77, line 13, corrects a typographical error by incorporating \leftrightarrow . It is commonly known in the art that a 1-site model for a single ligand binding to a single receptor is represented by a simple langmuir system, $A+B \leftrightarrow AB$. The amendment is further supported by U.S. Serial No. 09/603,825 (page 23, line 10), to which this application claims priority. Thus the above amendment does not introduce any new matter, and accordingly, the entry of the above amendment is respectfully requested.

The changes in the specification do not involve new matter and entry of them is respectfully requested. If a telephone interview would be of assistance in advancing the prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone her at the number provided below.

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No additional fee is deemed necessary in connection with the filing of this Amendment.
If any additional fees are necessary, the Patent Office is authorized to charge any
additional fee to Deposit Account No. 50-0306.

Respectfully submitted,

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MARKED-UP VERSION TO SHOW AMENDMENT OF SPECIFICATION

Please amend the specification at page 1, lines 5-10, to read as follows:

-- This application is a continuation-in-part of U.S. Serial No. 08/228,208, filed April 15, 1994, now U.S. Patent No. 6,090,914, issued July 18, 2000, which was a continuation-in-part of U.S. Serial No. 08/008, 898, filed January 22, 1993, now U.S. Patent No. 5,770,197, issued June 23, 1998, which was a continuation-in part of U.S. Serial No. 07/723,617, filed June 27, 1991, now abandoned; U.S. Serial No. 09/603,825, filed June 26, 2000, which was a continuation-in-part of U.S. Serial No. 09/014,761, filed January 28, 1998, which claims priority of U.S. Serial No. 60/036,594, filed January 31, 1997, now abandoned; and U.S. Serial No. 08/539,436, filed October 5, 1995, now U.S. Patent No. 6,132,992, issued October 17, 2000, [U.S. Serial No. 08/539, 436, filed October 19, 1995, and U.S. Serial No. not yet known, filed June 26, 2000, which is a continuation in part of U.S. Serial No. 09/014,761, filed January 28, 1998, which claims priority of U.S. Serial No. 60/036,549, filed January 28, 1997, now abandoned,] the contents of all of which are hereby incorporated by reference, in their entirety, into the present application. --.

Please amend the specification at page 4, lines 14-16, to read as follows:

-- Figure 3: Shows the nucleotide (SEQ ID NO.: 1) and complete amino acid sequence (SEQ ID NO.: 2) encoding human CTLA4 receptor fused to the oncostatin M signal peptide, and including the newly identified N-linked glycosylation site, as described in Example 1, infra. --.

Please amend the specification at page 6, lines 20-25, to read as follows:

-- Figure 17: Depicts sequencing alignment of CD28 and CTLA4 family members. Sequences of human (H) (SEQ ID NO.: 7), mouse (M) (SEQ ID NO.: 5), rat (R) (SEQ ID NO.: 6), and chicken (Ch) CD28 (SEQ ID NO.: 8) are aligned with human (SEQ ID NO.: 3) and mouse CTLA4 (SEQ ID NO.: 4). The signal peptides are underlined with a dashed line. The transmembrane domains are underlined with a solid line. The CDR-analogous regions

are noted. The dark shaded areas highlight complete conservation of residues while the light shaded areas highlight conservative amino acid substitutions in all family members.--

Please amend the specification at page 7, lines 11-12, to read as follows:

-- Figure 22: Depicts the nucleotide (SEQ ID NO.: 9) and amino acid sequence of a CTLA4Ig (SEQ ID NO.: 10) having wildtype extracellular domain of CTLA4. --

Please amend the specification at page 7, lines 15-17, to read as follows:

-- Figure 23: Depicts the nucleotide (SEQ ID NO.: 11) and amino acid (SEQ ID NO.: 12) sequences of L104EIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.--

Please amend the specification at page 7, lines 19-21, to read as follows:

-- Figure 24: Depicts the nucleotide (SEQ ID NO.: 13) and amino acid sequence (SEQ ID NO.: 14) of L104EA29YIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.--

Please amend the specification at page 7, lines 23-25, to read as follows:

-- Figure 25: Depicts the nucleotide (SEQ ID NO.: 15) and amino acid sequences (SEQ ID NO.: 16) of L104EA29LIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124. --

Please amend the specification at page 7, lines 27-29, to read as follows:

-- Figure 26: Depicts the nucleotide (SEQ ID NO.: 17) and amino acid sequences (SEQ ID NO.: 18) of L104EA29TIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124. --

Please amend the specification at page 8, lines 1-3, to read as follows:

-- Figure 27: Depicts the nucleotide (SEQ ID NO.: 19) and amino acid sequences (SEQ ID NO.: 20) of L104EA29Wlg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position -1 to aspartic acid at position +124.--

Please amend the specification at page 8, lines 22-25, to read as follows: Please replace the paragraph at page 9, lines 22-25, with the following rewritten paragraph:

-- Figure 37: [Figure 35] Depicts the results of a FACS assay, showing L104EIg and L104ES25RIg bind CHO cells stably transfected with human CD80 or CD86. A) L104EIg and L104ES25RIg bind to human CD80 CHO-transfected cells; B) L104EIg and L104ES25RIg bind to human CD86 CHO-transfected cells.--

Please amend the specification at page 11, lines 1-8, to read as follows:

-- One embodiment of a soluble CTLA4 has been deposited with the American Type Culture Collection (ATCC) in Manassas, Maryland, under the provisions of the Budapest Treaty on May 31, 1991 and has been accorded ATCC accession number: 68629. ATCC 68629 is DNA encoding *CTLA4*Ig. Additionally, the CTLA4Ig-24 CHO cell line has been deposited with the ATCC under the Budapest Treaty on May 31, 1991 and has been accorded accession number ATCC 10762. DNA encoding L104EA29YIg was submitted for deposit on June 19, 2000 with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209. [The ATCC accession number has not yet been assigned.] The DNA encoding L104EA29YIg has been accorded ATCC accession number PTA-2104. --

Please amend the specification at page 33, lines 2-21, to read as follows:

-- Because a signal peptide for CTLA4 was not identified in the CTLA4 gene, the N-terminus of the predicted sequence of CTLA4 was fused to the signal peptide of oncostatin M (Malik et al., Mol. and Cell. Biol. 9:2847 (1989)) in two steps using overlapping oligonucleotides. For the first step, the oligonucleotide, CTCAGTCTGGTCCTTGCACTCCTGTTCCAAGCATGGCGAGCATGGCAATGCACC

TGGCCCAGCC (SEQ ID NO.: 21) (which encoded the C terminal 15 amino acids from the oncostatin M signal peptide fused to the N terminal 7 amino acids of CTLA4) was used as forward primer, and TTTGGGCTCCTGATCAGAATCTGGGCACGGTTG (SEQ ID NO.: 22) (encoding amino acid residues 119-125 of the amino acid sequence encoding CTLA4 receptor and containing a Bcl I restriction enzyme site) as reverse primer. The template for this step was cDNA synthesized from 1 micro g of total RNA from H38 cells (an HTLV II infected T cell leukemic cell line provided by Drs. Salahudin and Gallo, NCI, Bethesda, MD). A portion of the PCR product from the first step was reamplified, using an overlapping forward primer, encoding the N terminal portion of the oncostatin M signal peptide and containing a Hind III restriction endonuclease site, CTAGCCACTGAAGCTTCACCAATGGGTGTACTGCTCACACAGAGGACGCTGCTCAGTCTGGTCCTTGCACTC (SEQ ID NO.: 23) and the same reverse primer. The product of the PCR reaction was digested with Hind III and Bcl I and ligated together with a Bcl I/Xba I cleaved cDNA fragment encoding the amino acid sequences corresponding to the hinge, CH2 and CH3 regions of IgC γ 1 into the Hind III/Xba I cleaved expression vector, CDM8 or Hind III/Xba I cleaved expression vector pLN. --.

Please amend the specification at page 33, lines 23-29, to read as follows:

-- A schematic map of the resulting CTLA4Ig fusion construct is shown in Figure 1. Sequences displayed in this figure show the junctions between CTLA4 (upper case letters, unshaded regions) and the signal peptide, SP, of oncostatin M (dark shaded regions), and the hinge, H, of IgC(gamma)1 (stippled regions). The amino acid in parentheses was introduced during construction. Asterisks (*) indicate cysteine to serine mutations introduced in the IgC γ hinge region. The immunoglobulin superfamily V-like domain present in CTLA4 is indicated, as are the CH2 and CH3 domains of IgC(gamma)1. --.

Please amend the specification at page 34, lines 21-27, to read as follows:

-- CTLA4Ig was purified by protein A chromatography from serum-free conditioned supernatants (Figure 2). Concentrations of CTLA4Ig were determined assuming an

extinction coefficient at 280 nm of 1.6 (experimentally determined by amino acid analysis of a solution of known absorbance). Molecular weight standards (lanes 1 and 3, Figure 2) and samples (1 micro grams) of CTLA4Ig (lanes 2 and 4) were subjected to SDS-PAGE (4-12% acrylamide gradient) under non-reducing conditions (- beta ME, lanes 1 and 2) or reducing conditions (+ beta ME, lanes 3 and 4) Proteins were visualized by staining with Coomassie Brilliant Blue. --.

Please amend the specification at page 35, lines 19-31, to read as follows:

-- Because the expression of CTLA4 receptor protein in human lymphoid cells has not been previously reported, it was necessary to locate a source of CTLA4 mRNA. cDNA was reverse transcribed from the total cellular RNA of H38 cells, as noted above, was used for cloning by PCR. For this purpose, the oligonucleotide, GCAATGCACGTGGCCCAGCCTGCTGTGGTAGTG (SEQ ID NO.: 24) (encoding the first 11 amino acids in the predicted coding sequence) was used as a forward primer, and TGATGTAACATGTCTAGATCAATTGATGGGAATAAAATAAGGCTG (SEQ ID NO.: 25) (homologous to the last 8 amino acids in CTLA4 and containing a Xba I site) as reverse primer. The template again was a cDNA synthesized from 1 micro gram RNA from H38 cells. Products of the PCR reaction were cleaved with the restriction endonucleases Nco I and Xba I and the resulting 316 bp product was gel purified. A 340 bp Hind III/Nco I fragment from the CTLA4Ig fusion described above was also gel-purified, and both restriction fragments were ligated into Hind III/Xba I cleaved CDM8 to form OMCTLA. --.

Please amend the specification at page 36, lines 21-25, to read as follows:

-- Receptor-immunoglobulin C gamma (IgC γ) fusion proteins B7Ig and CD28Ig were prepared as described by Linsley et al., in J. Exp. Med. 173:721-730 (1991), incorporated by reference herein. Briefly, DNA encoding amino acid sequences corresponding to the respective receptor protein (e.g. B7) was joined to DNA encoding amino acid sequences

corresponding to the hinge, CH2 and CH3 regions of human IgG₁. This was accomplished as follows. --

Please amend the specification at page 37, lines 6-25, to read as follows:

-- Plasmid Construction. Expression plasmids containing cDNA encoding CD28, (as described by Aruffo and Seed, Proc. Natl. Acad. Sci. USA 84:8573 (1987)), were provided by Drs. Aruffo and Seed (Mass General Hospital, Boston, MA). Plasmids containing cDNA encoding CD5, (as described by Aruffo, Cell 61:1303 (1990)), were provided by Dr. Aruffo. Plasmids containing cDNA encoding B7, (as described by Freeman et al., J. Immunol. 143:2714 (1989)), were provided by Dr. Freeman (Dana Farber Cancer Institute, Boston, MA). For initial attempts at expression of soluble forms of CD28 and B7, constructs were made (OMCD28 and OMB7) as described by Linsley et al., J. Exp. Med., *supra*, in which stop codons were introduced upstream of the transmembrane domains and the native signal peptides were replaced with the signal peptide from oncostatin M (Malik et al., Mol. Cell Biol. 9:2847 (1989)). These were made using synthetic oligonucleotides for reconstruction (OMCD28) or as primers (OMB7) for PCR. OMCD28, is a CD28 cDNA modified for more efficient expression by replacing the signal peptide with the analogous region from oncostatin M. CD28Ig and B7Ig fusion constructs were made in two parts. The 5' portions were made using OMCD28 and OMB7 as templates and the oligonucleotide, CTAGCCACTGAAGCTTCACCATGGGTGTTACTGCTCACAC (SEQ ID NO.: 26) (encoding the amino acid sequence corresponding to the oncostatin M signal peptide) as a forward primer, and either TGGCATGGGCTCCTGATCAGGCTTAGAAGGTCCGGGAAA (SEQ ID NO.: 27) or, TTTGGGCTCCTGATCAGGAAAATGCTCTTGCTTGGTTGT (SEQ ID NO.: 28) as reverse primers, respectively. Products of the PCR reactions were cleaved with restriction endonucleases (Hind III and BclI) as sites introduced in the PCR primers and gel purified.--

Please amend the specification at page 37, lines 27, through page 38, line 9, to read as follows:

-- The 3' portion of the fusion constructs corresponding to human IgC γ 1 sequences was made by a coupled reverse transcriptase (from Avian myeloblastosis virus; Life Sciences Associates, Bayport, NY)-PCR reaction using RNA from a myeloma cell line producing human-mouse chimeric mAb L6 (provided by Dr. P. Fell and M. Gayle, Bristol-Myers Squibb Company, Pharmaceutical Research Institute, Seattle, WA) as template. The oligonucleotide,

AAGCAAGAGCATTTTCCTGATCAGGAGCCCAAATCTTCTGACAAAACCTCACA-
CATCCCCACCGTCCCCAGCACCTGAACTCCTG (SEQ ID NO.: 29) was used as
forward primer, and

CTTCGACCAGTCTAGAAGCATCCTCGTGCGACCGCGAGAGC (SEQ ID NO.: 30)
as reverse primer. Reaction products were cleaved with BclI and XbaI and gel purified. Final constructs were assembled by ligating HindIII/BclI cleaved fragments containing CD28 or B7 sequences together with BclI/XbaI cleaved fragment containing IgC γ 1 sequences into HindIII/XbaI cleaved CDM8. Ligation products were transformed into MC1061/p3 *E. coli* cells and colonies were screened for the appropriate plasmids. Sequences of the resulting constructs were confirmed by DNA sequencing. --.

Please amend the specification at page 38, lines 17-24, to read as follows:

-- CD5Ig was constructed in identical fashion, using
CATTGCACAGTCAAGCTTCCATGCCCATGGGTTCTCTGGCCACCTTG (SEQ ID
NO.: 31) as forward primer and
ATCCACAGTGCAGTGATCATTGGATCCTGGCATGTGAC (SEQ ID NO.: 32) as
reverse primer. The PCR product was restriction endonuclease digested and ligated with the
IgC γ 1 fragment as described above. The resulting construct (CD5Ig) encoded a mature
protein having an amino acid sequence containing amino acid residues from position 1 to
position 347 of the sequence corresponding to CD5, two amino acids introduced by the

construction procedure (amino acids DQ), followed by DNA encoding amino acids corresponding to the IgC γ 1 hinge region. --.

Please amend the specification at page 39, lines 21-30, to read as follows:

-- Immunostaining and FACS^R Analysis. Transfected CHO or COS cells or activated T cells were analyzed by indirect immunostaining. Before staining, CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with murine mAbs 9.3 (Hansen et al., Immunogenetics 10:247 (1980)) or BB-1 (Yokochi et al., J. Immunol. 128:823 (1981)), or with Ig fusion proteins (all at 10 micrograms/ml in DMEM containing 10% FCS) for 1-2 h at 4 °C. Cells were then washed, and incubated for an additional 0.5-2h at 4 °C with a FITC-conjugated second step reagent (goat anti-mouse Ig serum for murine mAbs, or goat anti-human Ig C γ serum for fusion proteins (Tago, Inc., Burlingame, CA)). Fluorescence was analyzed on a FACS IV^R cell sorter (Becton Dickinson and CO., Mountain View, CA) equipped with a four decade logarithmic amplifier. --.

Please amend the specification at page 40, line 8, through page 41, line 5, to read as follows:

-- mAbs. Murine monoclonal antibodies (mAbs) 9.3 (anti-CD28) and G19-4 (anti-CD3), G3-7 (anti-CD7), BB-1 (anti-B7 antigen) and rat mAb 187.1 (anti-mouse kappa chain) have been described previously (Ledbetter et al., Proc. Natl. Acad. Sci. 84:1384-1388 (1987); Ledbetter et al., Blood 75:1531 (1990); Yokochi et al., supra) and were purified from ascites before use. The hybridoma producing mAb OKT8 was obtained from the ATCC, Rockville, MD, and the mAb was also purified from ascites before use. mAb 4G9 (anti-CD19) was provided by Dr. E. Engleman, Stanford University, Palo Alto, CA). Purified human-mouse chimeric mAb L6 (having human C γ 1 Fc portion) was a gift of Dr. P. Fell and M. Gayle (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA). --.

Please amend the specification at page 41, lines 7-15, to read as follows:

-- Immunostaining and FACS^R Analysis. Prior to staining, COS or CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with mAbs or Ig fusion proteins at 10 micro grams/ml in DMEM containing 10% FBS for 1-2 hr at 4 degrees C. Cells were then washed, and incubated for an additional 0.5-2 hrs at 4 degrees C with FITC-conjugated goat anti-mouse immunoglobulin or with FITC-conjugated goat anti-human Ig Cy serum (both from Tago, Burlingame, CA). When binding of both mAbs and Ig fusion proteins were measured in the same experiment, FITC-conjugated anti-mouse and anti-human second step reagents were mixed together before use. Fluorescence on a total of 10,000 cells was then analyzed by FACS^R. --.

Please amend the specification at page 44, lines 10-17, to read as follows:

-- Binding of CTLA4Ig on B7 Positive CHO cells. To further characterize the binding of CTLA4Ig and B7, the binding activity of purified CTLA4Ig on B7⁺ CHO cells and on a lymphoblastoid cell line (PM LCL) was measured in the experiment shown in Figure 5. Amplified transfected CHO cell lines and PM LCLs were incubated with medium only (no addition) or an equivalent concentration of human IgCy1-containing proteins (10 micro grams/ml) of CD5Ig, CD28Ig or CTLA4Ig. Binding was detected by FACS^R following addition of FITC-conjugated goat anti-human Ig second step reagents. A total of 10,000 stained cells were analyzed by FACS^R. --.

Please amend the specification at page 46, lines 14-19, to read as follows:

-- Primary mixed lymphocyte reaction (MLR) blasts were stimulated with irradiated T51 lymphoblastoid cells (LC) in the absence or presence of concentrations of murine mAb 9.3 Fab fragments, or B7Ig, CD28Ig or CTLA4Ig immunoglobulin Cy fusion proteins. Cellular proliferation was measured by [³H]-thymidine incorporation after 4 days and is expressed as the percentage of incorporation by untreated cultures (21,000 cpm). Figure 9 shows the mean of quadruplicate determinations (SEM \leq 10%). --.

Please amend the specification at page 48, lines 8-17, to read as follows:

-- These results demonstrate the first expression of a functional protein product of CTLA4 transcripts. CTLA4Ig, a fusion construct containing the extracellular domain of CTLA4 fused to an IgC_γ1 domain, forms a disulfide-linked dimer of M_r approximately 50,000 subunits. Because no interchain disulfides would be predicted to form in the Ig portion of this fusion, it seems likely that cysteines from CTLA4 are involved in disulfide bond formation. The analogous CD28Ig fusion protein (Linsley et al, supra, 1991) also contains interchain disulfide linkage(s). These results suggest that CTLA4 receptor, like CD28 (Hansen et al., Immunogenetics 10:247-260 (1980)), exists on the T cell surface as a disulfide linked homodimer. Although CD28 and CTLA4 are highly homologous proteins, they are immunologically distinct, because the anti-CD28 mAb, mAb 9.3, does not recognize CTLA4 (Figures 4 and 5). --

Please amend the specification at page 57, lines 20-23, to read as follows:

-- In addition, two mutants encoding the residues P103A and Y104A (MYPPAY (SEQ ID NO.: 40) and MYPPPA (SEQ ID NO.: 41), respectively) from the CD28Ig 99MYPPPY104 hexapeptide using CD28Ig as a template were also prepared by the same method. --.

Please amend the specification at page 57, lines 30, through page 58, line 1, to read as follows:

-- These primers encoded the following sequences:

CDM8FP: 5'-AATACGACTCACTATAGG (SEQ ID NO.: 33)

CDM8RP: 5'-CACCACACTGTATTAACC (SEQ ID NO.: 34)

Please amend the specification at page 58, line 29, through page 59, line 2, to read as follows:

-- HS7, HSS, and HS9 constructs were prepared by replacing a ~ 350 base-pair HindIII/HpaI 5' fragment of HS4, HS4-A, and HS4-B, respectively, with the equivalent cDNA fragment similarly digested from HS5 thus introducing the CDR1-like loop of CTLA4 into those hybrids already containing the CTLA4 CDR3-like region. --.

Please amend the specification at page 62, lines 29-31, to read as follows:

-- Several versions of the model with modified assignments of some residues to β -strands or loops were tested using 3D-profile analysis (Luthy et al., 1992, Nature 336:83-85) in order to improve the initial alignment of the CTLA4 extracellular region sequence with an IGSF variable fold. --

Please amend the specification at page 63, lines 12-15, to read as follows:

-- Regions of sequence conservation are scattered throughout the extracellular domains of these proteins with the most rigorous conservation seen in the hexapeptide MYPPPY (SEQ ID NO.: 35) motif located in the CDR3-like loop of both CTLA4 and CD28 (Figure 17). This suggests a probable role for this region in the interaction with a B7 antigen, e.g., B7-1 and B7-2. --.

Please amend the specification at page 73, lines 1-45, to read as follows:

-- TABLE B. Binding of CTLA4 and CD28 monoclonal antibodies to CTLA4Ig and CD28Ig mutant fusion proteins and to CTLA4/CD28Ig hybrid fusion proteins.

	<u>anti-CTLA4 mAbs</u>			<u>anti-CD28 mAb</u>
	7F8	11D4	10A8	9.3
<u>CTLA4Ig MUTANT FUSION PROTEIN</u>				
AYPPPY (SEQ ID NO.: 36)	+++	+++	+++	-
MAPPPY (SEQ ID NO.: 37)	++	+	++	-
MYAPPY (SEQ ID NO.: 38)	+	-	+	-
MYPAPY (SEQ ID NO.: 39)	+++	+++++	+++	-
MYPPAY (SEQ ID NO.: 40)	+++	-	+	-

MYPPPA (SEQ ID NO.: 41)	+++	++	+++	-
AAPPY (SEQ ID NO.: 42)	+	++	+++	-

CD28Ig MUTANT FUSION PROTEIN

MYPPAY (SEQ ID NO.: 40)	-	-	-	-
MYPPPA (SEQ ID NO.: 41)	-	-	-	+

CTLA4/CD28Ig HYBRID FUSION PROTEINS

HS1	-	-	-	-
HS2	-	-	-	+
HS3	-	-	-	-
HS4	-	-	-	+++
HS5	-	-	-	-
HS6	+	-	-	-
HS4-A	-	-	-	++
HS4-B	-	-	-	++
HS7	-	-	-	+++
HS8	-	+	-	+++
HS9	-	+	-	-
HS10	-	-	-	-
HS11	-	-	-	+
HS12	-	-	-	-
HS13	-	-	-	-
HS14	-	-	-	-
CTLA4Ig	+++	+++	+++	-
CD28Ig	-	-	-	+++

Antibody binding was rated from that seen for wild type protein (+++) to above background (+), and no detectable binding (-). --.

Please amend the specification at page 77, lines 12-17, to read as follows:

-- Experimental data were first fit to a model for a single ligand binding to a single receptor (1-site model, i.e., a simple langmuir system, $A+B \rightleftharpoons AB$), and equilibrium association constants ($K_d=[A] \cdot [B]/[AB]$) were calculated from the equation $R=R_{max} \cdot C/(K_d+C)$. Subsequently, data were fit to the simplest two-site model of ligand binding (i.e., to a receptor having two non-interacting independent binding sites as described by the equation $R=R_{max1} \cdot C/(K_{d1}+C)+R_{max2} \cdot C/(K_{d2}+C)$).--.

Please amend the specification at page 84, lines 23-28, to read as follows:

-- From tyrosine +23 to threonine +30, a pool of degenerate forward primers were generated having the following sequence:

5' CGA GGC ATC GCT AGC TTT GTG TGT GAG XXK XXK XXK XXK XXK XXK
XXK XXK GAG GTC CGG GTG ACA GT 3' (SEQ ID NO.: 43)

Where X = any of the four nucleotides A, T, G, or C and K = either T, G, or C

Each primer contained a Nhe I restriction enzyme cut site. --.

Please amend the specification at page 84, lines 30-32, to read as follows:

-- The reverse primer had the following sequence:

5' GGT TGC CGC ACA GAC TTC GGT CAC CTG GCT GTC AGC CTG CCG AAG
CAC TGT CAC CCG GA 3' (SEQ ID NO.: 44) --.

Please amend the specification at page 86, lines 24-31, to read as follows:

-- Five mutants were enriched through these 5 rounds of panning.

Mut 9	F-E-P-K-R-G-V-Q (SEQ ID NO.: 45)
Mut 19	W-D-Q-Y-T-G-Y-G (SEQ ID NO.: 46)
Mut 71	W-D-A-Y-R-N-Q-Q (SEQ ID NO.: 47)
Mut 45	Y-D-H-P-Y-D-G-Q (SEQ ID NO.: 48)
Mut 4	W-D-Q-H-V-S-R-R (SEQ ID NO.: 49)
CTLA4	Y-A-S-P-G-K-A-T (SEQ ID NO.: 50)

EXHIBIT 1

Copy of Notice to Comply
with Requirements for
Patent Applications
Containing Nucleotide
Sequence and/or Amino
Acid Sequence Disclosures

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING
NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 CFR 1.821 - 1.825 for the following reason(s):

☒ 1. This application clearly fails to comply with the requirements of 37 CFR 1.821 - 1.825. Applicant's attention is directed to these regulations, published at 1114 OG 37 May 15, 1990 and at 55 FR 18230, May 1, 1990.

☒ 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 CFR 1.821(c).

☐ 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 CFR 1.821(e).

☐ 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 CFR 1.822 and/or 1.823, as indicated on the attached copy of the marked-up "Raw Sequence Listing."

☐ 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A substitute computer readable form must be submitted as required by 37 CFR 1.825(d).

☐ 6. The paper copy of the "Sequence Listing" is not the same as the computer readable form of the "Sequence Listing" as required by 37 CFR 1.821(e).

☒ 7. Applicants should follow the format of the attached sample
Other: ~~statement if they request that the CRF filed in the patent~~
Applicant must provide: ~~application should be used to create a CRF~~
~~in this application.~~

☒ An initial or substitute computer readable form (CRF) copy of the "Sequence Listing"

☒ An initial or substitute paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification

☒ A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 CFR 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d)

For questions regarding compliance with these requirements, please contact:

For Rules Interpretation, call (703) 308-1123

For CRF submission help, call (703) 308-4212

For PatentIn software help, call (703) 557-0400

Please return a copy of this notice with your response.

EXHIBIT 2

Sequence Listing in paper
copy and Declaration
Pursuant to 37 C.F.R.
§1.821(f)

SEQUENCE LISTING

<110> Linsley, Peter S
Ledbetter, Jeffrey A
Bajorath, Jurgen
Peach, Robert J
Brady, William
Wallace, Philip
Damle, Nitin K

<120> SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

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Met Leu Arg Leu Leu Leu Ala Leu Asn Leu Phe Pro Ser Ile Gln Val
 1 5 10 15

Thr Gly Asn Lys Ile Leu Val Lys Gln Ser Pro Met Leu Val Ala Tyr
 20 25 30

Asp Asn Ala Tyr Asn Leu Ser Cys Lys Tyr Ser Tyr Asn Leu Phe Ser
 35 40 45
 Arg Glu Phe Arg Ala Ser Leu His Lys Gly Leu Asp Ser Ala Val Glu
 50 55 60
 Val Cys Val Val Tyr Gly Asn Tyr Ser Gln Gln Leu Gln Val Tyr Ser
 65 70 75 80
 Lys Thr Gly Phe Asn Cys Asp Gly Lys Leu Gly Asn Glu Ser Val Thr
 85 90 95
 Phe Tyr Leu Gln Asn Leu Tyr Val Asn Gln Thr Asp Ile Tyr Phe Cys
 100 105 110
 Lys Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys Ser
 115 120 125
 Asn Gly Thr Ile Ile His Val Lys Gly Lys His Leu Cys Pro Ser Pro
 130 135 140
 Leu Phe Pro Gly Pro Ser Lys Pro Phe Trp Val Leu Val Val Val Gly
 145 150 155 160
 Gly Val Leu Ala Cys Tyr Ser Leu Leu Tyr Thr Val Ala Phe Ile Ile
 165 170 175
 Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met
 180 185 190
 Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro
 195 200 205
 Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser
 210 215 220

<210> 8
 <211> 221
 <212> PRT
 <213> Gallus gallus

<400> 8

Met Leu Gly Ile Leu Val Val Leu Cys Leu Ile Pro Ala Ala Asp Val
1 5 10 15

Thr Glu Asn Lys Ile Leu Val Ala Gln Arg Pro Leu Leu Ile Val Ala
20 25 30

Asn Arg Thr Ala Thr Leu Val Cys Asn Tyr Thr Tyr Asn Gly Thr Gly
35 40 45

Lys Glu Phe Arg Ala Ser Leu His Lys Gly Thr Asp Ser Ala Val Glu
50 55 60

Val Cys Phe Ile Ser Trp Asn Met Thr Lys Ile Asn Ser Asn Ser Asn
65 70 75 80

Lys Glu Phe Asn Cys Arg Gly Ile His Asp Lys Asp Lys Val Ile Phe
85 90 95

Asn Leu Trp Asn Met Ser Ala Ser Gln Thr Asp Ile Tyr Phe Cys Lys
100 105 110

Ile Glu Ala Met Tyr Pro Pro Pro Tyr Val Tyr Asn Glu Lys Ser Asn
115 120 125

Gly Thr Val Ile His Tyr Arg Glu Thr Pro Ile Gln Thr Gln Glu Pro
130 135 140

Glu Ser Ala Thr Ser Tyr Trp Val Met Tyr Ala Val Thr Gly Leu Leu
145 150 155 160

Gly Phe Tyr Ser Met Leu Ile Thr Ala Val Phe Ile Ile Tyr Arg Gln
165 170 175

Lys Ser Lys Arg Asn Arg Tyr Arg Gln Ser Asp Tyr Met Asn Met Thr
180 185 190

Pro Arg His Pro Pro His Gln Lys Asn Lys Gly Tyr Pro Ser Tyr Ala
195 200 205

Pro Thr Arg Asp Tyr Thr Ala Tyr Arg Ser Trp Gln Pro
210 215 220

<210> 9

<211> 1152
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: CTLA4Ig

<400> 9

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atgggtgtac tgctcacaca gaggacgctg ctcagtctgg tccttgcaact cctgtttcca    60
agcatggcga gcatggcaat gcacgtggcc cagcctgctg tggactggc cagcagccga    120
ggcatcgcta gctttgtgtg tgagtatgca tctccaggca aagccactga ggtccgggtg    180
acagtgcctc ggcaggctga cagccaggtg actgaagtct gtgcggcaac ctacatgatg    240
gggaatgagt tgaccttcc agatgattcc atctgcacgg gcacctccag tggaaatcaa    300
gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg    360
gagctcatgt acccaccgcc atactacctg ggcataggca acggaacca gatttatgta    420
attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac    480
acatccccac cgtccccagc acctgaactc ctgggtggat cgtcagtctt cctcttcccc    540
ccaaaacca aggacacct catgatctcc cggacctctg aggtcacatg cgtggtggtg    600
gacgtgagcc acgaagacct tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg    660
cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg ggtggtcagc    720
gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggctctc    780
aacaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga    840
gaaccacagg tgtacacct gcccccctcc cgggatgagc tgaccaagaa ccaggtcagc    900
ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat    960
gggcagccgg agaacaacta caagaccag cctcccgctg tggactccga cggctccttc   1020
ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca   1080
tgctccgtga tgcattgagg tctgcacaac cactacacgc agaagagcct ctccctgtct   1140
ccgggtaaat ga                                     1152
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<210> 10
<211> 383
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: CTLA4Ig

<400> 10

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
35 40 45

Tyr Ala Ser Pro Gly Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg
50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Leu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
 260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
 275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
 340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375 380

<210> 11

<211> 1152

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: L104E1g

<400> 11

atgggtgtac tgctcacaca gaggacgctg ctcagtcttg tccttgcaact cctgtttcca 60

agcatggcga gcatggcaat gcaagtggcc cagcctgctg tggactggc cagcagccga 120

ggcatcgcta gctttgtgtg tgagtatgca tctccaggca aagccactga ggtccgggtg 180

acagtgcctc ggcaggctga cagccagggtg actgaagtct gtgcggcaac ctacatgatg 240

gggaatgagt tgaacttccc agatgattcc atctgcaagg gaacttcacg tggaaatcaa 300
 gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg 360
 gagctcatgt acccaccgcc atactacgag ggcataaggca acggaaccca gatttatgta 420
 attgatccag aaccgtgccg agattctgat caggagccca aatcttctga caaaactcac 480
 acatcccccac cgtcccccagc acctgaactc ctgggggggat cgtcagtctt cctcttcccc 540
 ccaaaaccca aggacaccct catgatctcc cggacccttg aggtcacatg cgtggtggtg 600
 gacgtgagcc acgaagacct tgaggtcaag ttcaactggc acgtggacgg cgtggaggtg 660
 cataatgcc aacaaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 720
 gtcttcaccg tctgcacca ggactggctg aatggcaagg agtacaagtg caaggctctc 780
 aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840
 gaaccacagg tgtacaccct gcccccctcc cgggatgagc tgaccaagaa ccaggtcagc 900
 ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960
 gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc 1020
 ttctctaca gcaagctcac cgtggacaag agcagggtggc agcaggggaa cgtcttctca 1080
 tgctccgtga tgcattgagc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140
 ccgggtaaat ga 1152

<210> 12
 <211> 383
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EIg

<400> 12

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
 1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
 20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
 35 40 45

Tyr Ala Ser Pro Gly Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg

50

55

60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
 340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375 380

<210> 13
 <211> 1152
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EA29YIg

<400> 13
 atgggtgtac tgctcacaca gaggacgctg ctcaagtctgg tccttgcaact cctgtttcca 60
 agcatggcga gcatggcaat gcacgtggcc cagcctgctg tggactaggc cagcagccga 120
 ggcacgcta gctttgtgtg tgagtatgca tctccaggca aatatactga ggtccgggtg 180
 acagtgttc ggcaggctga cagccagggtg actgaagtct gtgcggcaac ctacatgatg 240
 gggaatgagt tgaccttctt agatgattcc atctgcacgg gcacctccag tggaaatcaa 300
 gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg 360
 gagctcatgt acccacggcc ataactacgag ggcataggca acggaaccca gatttatgta 420
 attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480
 acatccccac cgtccccagc acctgaactc ctgggggggat cgtcagtctt cctcttcccc 540
 ccaaaaccca aggacacct catgatctcc cggacctctg aggtcacatg cgtsgtggtg 600
 gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggagggtg 660

cataatgcc aagacaaagcc ggggaggag cagtacaaca gcacgtaccg tgggtcagc 720
 gtcctcaccg tctgtcacca ggactggctg aatggcaagg agtacaagtg caaggctctc 780
 aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840
 gaaccacagg tgtacacctt gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900
 ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960
 gggcagccgg agaacaacta caagaccacg cctcccgctg tggactccga cggctccttc 1020
 ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080
 tgctccgtga tgcattgagc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140
 ccgggtaaat ga 1152

<210> 14
 <211> 383
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: L104EA29YIg

<400> 14

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
 1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
 20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
 35 40 45

Tyr Ala Ser Pro Gly Lys Tyr Thr Glu Val Arg Val Thr Val Leu Arg
 50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
 65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
 85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
 100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
 115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
 130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
 145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
 165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
 210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
 260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
 275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg

340

345

350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 370 375 380

<210> 15

<211> 1152

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: L104EA29LIg

<400> 15

atgggtgtac tgctcacaca gaggacgctg.ctcagttctgg tccttgcaact cctgtttcca 60
 agcatggcga gcatggcaat gcacgtggcc cagcctgctg tgggtactggc cagcagccga 120
 ggcacgccta gctttgtgtg tgagtatgca tctccaggca aattgactga ggtccgggtg 180
 acagtgtctc ggcaggctga cagccagggtg actgaagtct gtgcggcaac ctacatgatg 240
 gggaatgagt tgaccttctt agatgatctc atctgcacgg gcacctccag tggaaatcaa 300
 gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg 360
 gagtcatgt acccaccgcc atactacgag ggcataggca acggaacca gatttatgta 420
 attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480
 acatccccac cgtccccagc acctgaactc ctggggggat cgtcagtctt cctcttcccc 540
 ccaaaacca aggacaccct catgatctcc cggaccctg aggtcacatg cgtgggtggg 600
 gacgtgagcc acgaagaccc tgagggtcaag ttcaactggg acgtggacgg cgtggaggtg 660
 cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtgggtcagc 720
 gtcttcaccg tcttgacca ggactggctg aatggcaagg agtacaagtg caaggctctcc 780
 aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840
 gaaccacagg tgtacaccct gccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900
 ctgacctgcc tgggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaac 960
 gggcagccgg agaacaacta caagaccag cctcccgtgc tggactccga cggctccttc 1020
 ttctctaca gcaagctcac cgtggacaag agcagggtggc agcaggggaa cgtcttctca 1080
 tgctccgtga tgcattgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140

cggggtaaat ga

1152

<210> 16
<211> 383
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: L104EA29LIg

<400> 16

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
35 40 45

Tyr Ala Ser Pro Gly Lys Leu Thr Glu Val Arg Val Thr Val Leu Arg
50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
370 375 380

<210> 17
<211> 1152
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: L104EA29TIg

<400> 17

atgggtgtac tgctcacaca gaggacgctg ctcagtcctg tccttgcaact cctgtttcca	60
agcatggcga gcattggcaat gcacgtggcc cagcctgctg tggacttggc cagcagccga	120
ggcatcgcta gctttgtgtg tgagtatgca tctccaggca aaactactga ggtccgggtg	180
acagtgcctc ggcaggctga cagccagggt actgaagtct gtgcggcaac ctacatgatg	240
gggaatgagt tgaccttctt agatgattcc atctgcacgg gcacctccag tggaaatcaa	300
gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaagggtg	360
gagctcatgt acccaacgcc atactacgag ggcataggca acggaaccca gatttatgta	420
attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac	480
acatccccac cgtccccagc acctgaactc ctggggggat cgtcagtcct cctcttcccc	540
ccaaaaccca aggacacct catgatctcc cggacccctg aggtcacatg cgtggtggtg	600
gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggagggtg	660
cataatgcc aacaaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc	720
gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggctctcc	780
aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga	840
gaaccacagg tgtacacct gccccatcc cgggatgagc tgaccaagaa ccaggtcagc	900
ctgacctgcc tggtcaaaagg cttctatccc agcgacatcg cctgggagtg ggagagcaat	960
gggcagccgg agaacaacta caagaccag cctcccgctg tggactccga cggctccttc	1020
ttctctaca gcaagctcac cgtggacaag agcagggtggc agcaggggaa cgtcttctca	1080
tgctccgtga tgcattgagc tctgcacaac cactacacgc agaagagcct ctcctgtct	1140
ccgggtaaat ga	1152

<210> 18

<211> 383

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: L104EA29TIg

<400> 18

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala

1

5

10

15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
35 40 45

Tyr Ala Ser Pro Gly Lys Thr Thr Glu Val Arg Val Thr Val Leu Arg
50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
370 375 380

<210> 19
<211> 1152
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EA29WIg

<400> 19
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ggcagcgcga gcttctgtgtg tgagtatgca tctccaggca atgggactga ggtccgggtg 180
acagtgcctc ggcaggctga cagccaggtg actgaagtct gtgcggcaac ctacatgatg 240
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 ccaaaaccca aggacacct catgatctcc cggacccttg aggtcacatg cgtgggtggtg 600
 gacgtgagcc acgaagaccc tgaggtcaag ttcaactggg acgtggacgg cgtggaggtg 660
 cataatgcca agacaaagcc gggggaggag cagtacaaca gcacgtaccg tgtggtcagc 720
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 35 40 45

Tyr Ala Ser Pro Gly Lys Trp Thr Glu Val Arg Val Thr Val Leu Arg
 50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
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Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu

290

295

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Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
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325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
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<210> 45
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<210> 46
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	Peter S. Linsley, et al.	
Serial No.	09/609,915	Examiner: E. M. Lazar-Wesley, Ph.D.
Filed	July 3, 2000	Group Art Unit: 1646
For	SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF	

55 S. Lake Avenue, Suite 710
Pasadena, California 91101
September 9, 2002

Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

**COMMUNICATION IN RESPONSE TO THE AUGUST 7, 2002
COMMUNICATION FROM THE EXAMINER**

This Communication is submitted in response to a Communication, dated August 7, 2002 from the Examiner issued in connection with the above-identified application. In accordance with the Notice, the deadline for submitting the response was September 7, 2002. However, since September 7, 2002, fell on a Saturday, a response filed the next business day, namely September 9, 2002, is considered timely. Accordingly, this response is being timely filed.

In the Notice, the Patent Office states that the reply filed April 22, 2002 to the sequence letter mailed March 15, 2002 is not fully responsive to the prior Office Action for the following reasons:

1. The specification does not comply with 37 C.F.R. 1.821 (d); and
2. The declaration pursuant to 37 C.F.R. 1.821(f) filed April 22, 2002, is deficient for failing to state that the paper and computer readable copy of sequence listings do not include new matter.

As requested by the Examiner, Applicants provide herein a preliminary amendment that incorporates sequence identifiers (SEQ ID NOs) in the claims (Exhibit 1).

With regards to the declaration, Applicants wish to point out that 37 C.F.R. §1.821(f) does not require that the declaration include the language "the paper copy and CRF of sequence listing do not include new matter." It is permissible to include that statement in the communication as shown in Applicants' responses filed April 15 and May 9, 2002. However, in order to further the prosecution of the subject application, Applicants provide herein a declaration that includes a statement that the paper and computer readable copy of sequence listings do not include new matter (Exhibit 2).

Applicants: Peter S. [redacted] et al.
U.S. Serial No. 09/609,915
Filed: July 3, 2000
Page 3

No fee is deemed necessary in connection with the filing of this Response. However, if any fee is necessary, the Patent Office is authorized to charge any additional fee to Deposit Account No. 50-0306.

Respectfully submitted,

Sarah B. Adriano

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SaraLynn Mandel
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Attorneys for Applicants
Mandel & Adriano
55 S. Lake Avenue, Suite 710
Pasadena, California 91101
(626) 395-7801
Customer No. 26,941

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley, et al.

Serial No. : 09/609,915

Examiner: E. M. Lazar-Wesley, Ph.D.

Filed : July 3, 2000

Group Art Unit: 1646

For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES
THEREOF

55 S. Lake Avenue, Suite 710
Pasadena, California 91101
September 9, 2002

Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

PRELIMINARY AMENDMENT

Please amend the subject application as follows.

In the Claims:

Please cancel claim 10 without prejudice. Additionally, please amend the claims as follows:

- 2. (Amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region S25-R33 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 51-59), and wherein the mutation is a substitution of any amino acid beginning with serine at position +25 of Figure 3 (SEQ ID NO.: 2, at position 51) and ending with lysine at position +28 of Figure 3 (SEQ ID NO.: 2, at position 54) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine,

glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. --

- 3. (Amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises a mutation in a region S25-R33 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 51-59), and wherein the mutation is a substitution of alanine at position +29 of Figure 3 (SEQ ID NO.: 2, at position 55) with any of arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, or valine. --
- 4. (Amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region S25-R33 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 51-59), and wherein the mutation is a substitution of any amino acid beginning with threonine at position +30 of Figure 3 (SEQ ID NO.: 2, at position 56) and ending with arginine at position +33 of Figure 3 (SEQ ID NO.: 2, at position 59) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.--
- 5. (Amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region E95-G107 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 121-133), and wherein the mutation is a substitution of any amino acid beginning with glutamic acid at position +95 of Figure 3 (SEQ ID NO.: 2, at position 121) and ending with leucine at position +96 of Figure 3 (SEQ ID NO.: 2, at position 122) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine,

methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. --

- 6. (Amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region E95-G107 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 121-133), and wherein the mutation is a substitution of any amino acid beginning with methionine at position +97 of Figure 3 (SEQ ID NO.: 2, at position 123) and ending with tyrosine at position +103 of Figure 3 (SEQ ID NO.: 2, at position 129) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. --
- 7. (Amended) The soluble CTLA4 mutant molecule of claim 6, wherein the mutation is a substitution of tyrosine at position +103 of Figure 3 (SEQ ID NO.: 2, at position 129) with a different amino acid selected from a group consisting of arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, and valine. --
- 8. (Amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region E95-G107 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 121-133), and wherein the mutation is a substitution of any amino acid beginning with leucine at position +104 of Figure 3 (SEQ ID NO.: 2, at position 130) and ending with glycine at position +107 of Figure 3 (SEQ ID NO.: 2, at position 133) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine,

methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. --

- 9. (Amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region N108-I115 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 134-141), and wherein the mutation is a substitution of any amino acid beginning with asparagine at position +108 of Figure 3 (SEQ ID NO.: 2, at position 134) and ending with isoleucine at position +115 of Figure 3 (SEQ ID NO.: 2, at position 141) with a different amino acid selected from a group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. --
- 20. (Amended) The method of claim 19, wherein the soluble CTLA4 mutant molecule is any of L104EA29L (SEQ ID NO.: 16), L104EA29T (SEQ ID NO.: 18), or L104EA29W (SEQ ID NO.: 20).

REMARKS

Claims 1-25 were pending. Applicants have canceled claim 10 without prejudice. Additionally, Applicants have amended claims 2-9, and 20. Accordingly, claims 1-9, and 11-25 are presently pending.

The amendments to claims 2-9, and 20 are made to provide SEQ ID NOs in the claims and correct typographical errors. Additionally, claim 9 is amended to incorporate the contents of claim 10, and claim 10 is canceled. The amendments to correct typographical errors and incorporate SEQ ID NOs in the claims do not introduce any new matter and

are supported by the disclosure as originally filed. Accordingly, entry of these amendments is respectfully requested.

Additionally, claim 5 is amended to correct a typographical error by correcting the amino acid at position +96 from "lysine" to "leucine". The amendment to correct the typographical error in claim 5 does not introduce new matter and is supported by the specification (Figure 3) as originally filed. Accordingly, entry of the amendment to claim 5 is respectfully requested.

The changes in the specification do not involve new matter and entry of them is respectfully requested. If a telephone interview would be of assistance in advancing the prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone her at the number provided below.

No additional fee is deemed necessary in connection with the filing of this Amendment. If any additional fees are necessary, the Patent Office is authorized to charge any additional fee to Deposit Account No. 50-0306.

Respectfully submitted,

Sarah B. Adriano

Sarah B. Adriano
Registration No. 34,470
SaraLynn Mandel
Registration No. 31,853
Attorneys for Applicants
Mandel & Adriano
55 S. Lake Avenue, Suite 710
Pasadena, California 91101
Tel: (626) 395-7801
Customer No. 26,941

MARKED-UP VERSION TO SHOW AMENDMENT OF SPECIFICATION

Please amend the claims to read as follows:

- 2. (Amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 [has] comprises one or more mutations in a region S25-R33 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 51-59), and wherein the mutation is a substitution of any amino acid beginning with [of] serine at position +25 of Figure 3 (SEQ ID NO.: 2, at position 51) and ending with [through] lysine at position +28 of Figure 3 (SEQ ID NO.: 2, at position 54) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. --
- 3. (Amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 [has] comprises a [one or more] mutation[s] in a region S25-R33 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 51-59), and wherein the mutation is a substitution of alanine at position +29 of Figure 3 (SEQ ID NO.: 2, at position 55) with any of arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, or valine. --
- 4. (Amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 [has] comprises one or more mutations in a region S25-R33 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 51-59), and wherein the mutation is a substitution of any amino acid beginning with [of] threonine at position +30 of Figure 3 (SEQ ID NO.: 2, at position 56) and ending with [through] arginine at position +33 of Figure 3 (SEQ ID NO.: 2, at position 59) with a different amino acid selected from alanine, arginine, asparagine,

aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.--

- 5. (Amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 [has] comprises one or more mutations in a region E95-G107 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 121-133), and wherein the mutation is a substitution of any amino acid beginning with [of] glutamic acid at position +95 of Figure 3 (SEQ ID NO.: 2, at position 121) and ending with [through] leucine at position +96 of Figure 3 (SEQ ID NO.: 2, at position 122) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. --
- 6. (Amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 [has] comprises one or more mutations in a region E95-G107 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 121-133), and wherein the mutation is a substitution of any amino acid beginning with [of] methionine at position +97 of Figure 3 (SEQ ID NO.: 2, at position 123) and ending with [through] tyrosine at position +103 of Figure 3 (SEQ ID NO.: 2, at position 129) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. --
- 7. (Amended) The soluble CTLA4 mutant molecule of claim [5] 6, wherein the mutation is a substitution of tyrosine at position +103 of Figure 3 (SEQ ID NO.: 2, at position 129) with a different amino acid selected from a group consisting of arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine,

histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, [tyrosine,] and valine. --

- 8. (Amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 [has] comprises one or more mutations in a region E95-G107 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 121-133), and wherein the mutation is a substitution of any amino acid beginning with [of] leucine at position +104 of Figure 3 (SEQ ID NO.: 2, at position 130) and ending with [through] glycine at position +107 of Figure 3 (SEQ ID NO.: 2, at position 133) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. --
- 9. (Amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 [has] comprises one or more mutations in a region N108-I115 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 134-141), and wherein the mutation is a substitution of any amino acid beginning with asparagine at position +108 of Figure 3 (SEQ ID NO.: 2, at position 134) and ending with [at] isoleucine at position +115 [(N108-I115)] of Figure 3 (SEQ ID NO.: 2, at position 141) with a different amino acid selected from a group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. --
- 20. (Amended) The method of claim 19, wherein the soluble CTLA4 mutant molecule is any of L104EA29L (SEQ ID NO.: 16), L104EA29T (SEQ ID NO.: 18), or L104EA29W (SEQ ID NO.: 20).

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley et al.
Serial No : 09/609,915
Filed : July 3, 2000
For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES
THEREOF

55 S. Lake Avenue, Suite 710
Pasadena, California 91101
September 6, 2002

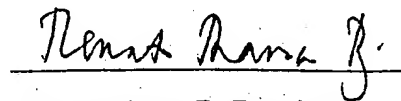
Assistant Commissioner for Patents
Box Sequence
Washington, D.C. 20231

SIR:

DECLARATION PURSUANT TO 37 C.F.R. §1.821(f)

I hereby declare that the content of the paper and computer readable copies of the Sequence Listing initially submitted on April 12, 2002 and the substitute Sequence Listing submitted on May 9, 2002 for the subject patent application in accordance with 37 C.F.R. §1.821(c) and (e), respectively, are the same. I further hereby state that the paper copy and computer readable copies of the Sequence Listings submitted on April 12, 2002 and May 9, 2002 contain no new matter pursuant to 37 C.F.R. 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d), and are supported by the specification as originally filed.

Respectfully submitted,


Renato Marco P. Domingo

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley, et al.

Serial No. : 09/609,915

Examiner: E. M. Lazar-Wesley, Ph.D.

Filed : July 3, 2000

Group Art Unit: 1646

For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES
THEREOF

55 South Lake Ave., Suite 710
Pasadena, California 91101
November 4, 2002

Assistant Commissioner for Patents
Washington, D.C. 20231

Madam/Sir:

**AMENDMENT IN RESPONSE
TO THE OCTOBER 2, 2002 OFFICE ACTION**

This Amendment is submitted in response to the Office Action dated October 2, 2002, issued by the U.S. Patent and Trademark office in connection with the above-identified patent application. A response to the Office Action was due November 2, 2002. However, since November 2, fell on a Saturday, a response filed the next business day, namely Monday, November 4, 2002, will be considered timely. Accordingly, this Amendment is being timely filed.

RESTRICTION REQUIREMENT

In the Office Action, the Patent Office is requiring restriction under 35 U.S.C. §121 to one of the following Groups of inventions:

- Group I: Claims 1-9 and 11-18 are directed to soluble CTLA4 mutant molecules, soluble CTLA4 mutant fusion molecules, nucleic acid molecules encoding the soluble CTLA4 mutant molecules, and methods for producing the soluble CTLA4 mutant molecules;
- Group II: Claims 19-22 are directed to methods for regulating a T cell interaction with a CD80 and/or CD86 positive cell; and
- Group III: Claims 23-25 are directed to methods for treating immunoproliferative diseases.

TRAVERSAL

Applicants hereby confirm election of the invention of Group I with traverse.

Reconsideration of the Restriction Requirement is requested for the following reasons:

Applicants point out that under MPEP §803, there are two criteria for a proper requirement for restriction, namely: (1) the invention must be independent and distinct; AND (2) there must be serious burden on the Examiner for restriction to be required.

Applicants respectfully contend that the first requirement of §803 has not been met, since the claims of Groups II and III depend, directly or indirectly, upon the claims of Group I. Specifically, Applicants contend that the methods for regulating T cell interaction recited in Group II are not independent of the molecules of Group I since the methods of Group II require administration of the soluble CTLA mutant molecules of Group I. Applicants also contend that the methods for treating immunoproliferative diseases recited in Group

III are not independent of the molecules of Group I since the methods of Group III require administration of the soluble CTLA mutant molecules of Group I.

Therefore, the invention in Groups I, and II and III, are not independent and distinct. Accordingly, the criteria for requiring the restriction has not been met.

Applicants respectfully contend that the second requirement of §803 has also not been met. The Patent Office has not demonstrated a serious burden for searching the art of Groups I-III. Each of the claims of Groups I and II are classified in the same class. Therefore, the art with respect to the claims in Groups I and II overlap. The Examiner can perform a search on the entire application without serious burden. Thus, search of the art with regard to the invention of Groups I and II would not place an undue burden on the Examiner. Moreover, separate prosecution of these claims would be unnecessarily duplicative and thus wasteful of Patent Office resources. Therefore, under MPEP Section 803, the instant claims do not require restriction.

Applicants submit that claims of Groups I, II and III should properly be examined together for the reasons discussed above. Applicants respectfully request that the Examiner reconsider and withdraw the Restriction Requirement as these claims.

Conclusion

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone her at the number provided below.

Applicants: Peter S. [redacted]nsley, et al.
U.S. Serial No. 09/609,915
Filed: July 3, 2000
Page: 4

No fee is deemed necessary in connection with the filing of this Amendment. If any fee is necessary, the Patent Office is authorized to charge any additional fee to Deposit Account No. 50-0306.

Respectfully submitted,

Sarah B. Adriano

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Patent Practitioners for Applicants
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley, et al.
Serial No. : 09/609,915 Examiner: Lorraine Spector, Ph.D.
Filed : July 3, 2000 Group Art Unit: 1647
For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

55 South Lake Ave., Suite 710
Pasadena, California 91101
June 2, 2004

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Madam/Sir:

**AMENDMENT IN RESPONSE TO THE MARCH 2, 2004 OFFICE ACTION
AND PETITION FOR TWO-MONTH EXTENSION OF TIME**

This Amendment is submitted in response to the Office Action dated March 2, 2004, issued by the U.S. Patent and Trademark Office in connection with the above-identified patent application. A one-month period for reply was set, making April 2, 2004, the deadline for filing a response to the Office Action. A two-month extension of time for responding to the Office Action is hereby requested. The required fee for this two-month extension of time is FOUR HUNDRED TWENTY DOLLARS, \$420.00 for large entity. Applicants hereby enclose a check including the amount of \$420.00 to cover the filing fee. A response to the Office Action with a two-month extension of time is now due June 2, 2004. Accordingly, this Amendment is being timely filed.

Please amend the subject application as follows:

In the claims:

In compliance with the practice guidelines for making amendments, Applicants present all pending claims with status indicators and submit new claims 26-28. Please cancel claims 3-4 and 6-8 without prejudice to pursue the subject matter of these claims in another application to be filed in the future.

Please amend claims 2, 5, 9, 12 and 17 as follows:

1. (Originally-filed) A soluble CTLA4 mutant molecule having the extracellular domain of CTLA4 which binds CD80 or CD86.
2. (Currently amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region S25-R33 of CTLA4 of Figure 3 (SEQ ID NO:2, at positions 51-59), and wherein the mutation is a substitution of any amino acid beginning with serine at position +25 of Figure 3 (SEQ ID NO:2, at position 51) and ending with ~~lysine~~arginine at position +28~~+33~~ of Figure 3 (SEQ ID NO:2, at position ~~54~~59) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.
3. (cancelled)
4. (cancelled)

5. (Currently amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region E95-G107 of CTLA4 of Figure 3 (SEQ ID NO.:2, at positions 121-133), and wherein the mutation is a substitution of any amino acid beginning with glutamic acid at position +95 of Figure 3 (SEQ ID NO.:2, at position 121) and ending with ~~leucine~~ glycine at position ~~+96~~ +107 of Figure 3 (SEQ ID NO.:2, at position ~~122-133~~) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.
6. (cancelled)
7. (cancelled)
8. (cancelled)
9. (Currently amended) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region N108-I115 of CTLA4 of Figure 3 (SEQ ID NO.:2, at positions 134-141), and wherein the mutation is a substitution of any amino acid beginning with asparagine at position +108 of Figure 3 (SEQ ID NO.:2, at position 134) and ending with isoleucine at position +115 of Figure 3 (SEQ ID NO.:2, at position 141) with a different amino acid selected from a group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.
10. (cancelled)

11. (Originally-filed) The soluble CTLA4 mutant molecule of claim 1 further comprising an amino acid sequence which alters the solubility, affinity or valency of the soluble CTLA4 mutant molecule for binding to CD80 or CD86.
12. (Currently amended) The soluble CTLA4 mutant molecule of claim 14-11, wherein the amino acid sequence comprises a human immunoglobulin constant region.
13. (Originally-filed) A nucleic acid molecule comprising a nucleotide sequence encoding the amino acid sequence corresponding to the soluble CTLA4 mutant molecule of claim 1.
14. (Originally-filed) A vector comprising the nucleotide sequence of claim 13.
15. (Originally-filed) A host vector system comprising the vector of claim 14 in a suitable host cell.
16. (Originally-filed) The host vector system of claim 15, wherein the suitable host cell is a prokaryotic cell or a eukaryotic cell.
17. (Currently amended) A method for producing a soluble CTLA4 mutant protein comprising growing the host vector system of claim 16 so as to produce the protein in the host cell and recovering the protein so produced.
18. (Originally-filed) A soluble CTLA mutant protein produced by the method of claim 17.
19. (Withdrawn) A method for regulating a T cell interaction with a CD80 and/or CD86 positive cell comprising contacting the CD80 and/or CD86 positive cell with the soluble CTLA4 mutant molecule of claim 1 so as to regulate the T cell interaction.

20. (Withdrawn) The method of claim 19, wherein the soluble CTLA4 mutant molecule is any of L104EA29L (SEQ ID NO.: 16), L104EA29T (SEQ ID NO.: 18), or L104EA29W (SEQ ID NO.: 20).
21. (Withdrawn) The method of claim 19, wherein the CD80 and/or CD86 positive cell is an antigen presenting cell.
22. (Withdrawn) The method of claim 19, wherein the interaction of the CTLA4-positive T cells with the CD80 and CD86 positive cells is inhibited.
23. (Withdrawn) A method for treating immunoproliferative diseases mediated by T cell interactions with B7 positive cells comprising administering to a subject the soluble CTLA4 mutant molecule of claim 1, in an amount effective to regulate T cell interactions with said B7 positive cells.
24. (Withdrawn) The method of claim 23, wherein said T cell interactions are inhibited.
25. (Withdrawn) The method of claim 23, wherein the immunoproliferative disease is graft versus host disease.

Please add new claims 26-28 as follows:

26. (New) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises amino acid residues from about position 93 to about position 124 of Figure 3 (SEQ ID NO:2 beginning at position 119 and ending at position 150) joined to amino acid residues from about position 1 to about position 94 of the amino acid sequence corresponding to the extracellular domain of CD28.

27. (New) The soluble CTLA4 mutant molecule of claim 1, wherein in the extracellular domain of CTLA4, the second proline in the amino acid motif MYPPPY (SEQ ID NO:35) is replaced with alanine.
28. (New) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises:
- one or more mutations in a region S25-R33 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 51-59), and wherein the mutation is a substitution of any amino acid beginning with serine at position +25 of Figure 3 (SEQ ID NO.: 2, at position 51) and ending with arginine at position +33 of Figure 3 (SEQ ID NO.: 2, at position 59) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine; and
 - one or more mutations in a region E95-G107 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 121-133), and wherein the mutation is a substitution of any amino acid beginning with glutamic acid at position +95 of Figure 3 (SEQ ID NO.: 2, at position 121) and ending with glycine at position +107 of Figure 3 (SEQ ID NO.: 2, at position 133) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

REMARKS

Claims 1-9 and 11-25 were pending. Claims 1-9 and 11-18 were elected with traverse. Claims 3, 4, 6-8 are cancelled herein. New claims 26-28 have been added. Accordingly, claims 1-2, 5, 9, 11-18 and 26-28 are being examined. Entry of these claims is requested.

Support for amended claim 2 may be found in the originally filed claim 2 and Figure 3. Support for amended claim 5 may be found in the originally filed claim 5 and Figure 3.

The amendment to claim 9 merely corrects a typographical error by amending "SEQ ID NO.:2" to "SEQ ID NO:2". No new matter is added. The amendment to claim 12 merely corrects the dependency of the claim. No new matter is added. The amendment to claim 17 merely corrects a typographical error by amending "CTLA" to "CTLA4". No new matter is added.

Support for new claim 26 may be found in the specifications of:

1. U.S. Serial No. 07/723,617, filed June 27, 1991 in originally filed Figure 3, SEQ ID NO:13 and SEQ ID NO:14; and at page 13, lines 3-6 and at page 15, lines 15-18.
2. U.S. Serial No. 08/228,208, filed April 15, 1994, in originally filed Figure 3, SEQ ID NO:13 and SEQ ID NO:14; and at page 20, lines 10-13 and at page 22, lines 20-22.
3. U.S. Serial No. 09/609,915, filed June 3, 2000 (subject application), in originally filed Figure 3, SEQ ID NO:2, and at page 58, lines 17-21, and at page 71, Table A.

Support for new claim 27 is found in the specifications of:

1. U.S. Serial No. 08/228,208, filed April 15, 1994, in originally filed Figure 3 and Table II at page 77; and at page 62, lines 1-4.
2. U.S. Serial No. 09/609,915, filed June 3, 2000 (subject application), in originally filed Table B at page 73, Figure 3 and SEQ ID NO:35; and at page 57, lines 16-18.

Support for new claim 28 is found in the specifications of:

1. U.S. Serial No. 08/228,208, filed April 15, 1994, at page 63, lines 16-35; page 64, lines 1-8; and at page 62, lines 1-4; Table I at page 76.
2. U.S. Serial No. 09/609,915, filed June 3, 2000 (subject application), at page 14, lines 12-22; page 69; Table A at page 71.

The sequences in Figures 3 of both U.S. Serial No. 07/723,617 and U.S. Serial No. 08/228,208 are identical to the sequence in Figure 3 in the subject application. Only the numbering is different. For example, in Figure 3 of the subject application, methionine is located at position +1; whereas in Figure 3 of U.S. Serial No. 07/723,617 and U.S. Serial No. 08/228,208, alanine is located at position +1.

Accordingly, the amendments to claims 2, 5, 9, 12 and 17 and the addition of new claims 26-28, do not introduce new matter, and their entry is respectfully requested.

RESTRICTION REQUIREMENT

The Patent Office is requiring a species election to one of the patentably distinct species of the chosen group for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claims 1 and 11-18 are generic.

ELECTION WITH TRAVERSE

Applicants hereby elect, with traverse, the species of claim 26. Applicants reserve the right to present additional species upon indication of an allowable generic claim.

Support for claim 26 is found in U.S. Serial No. 07/723,617 ('617 application), filed June 27, 1991 and thus claims priority to the '617 application.

CONCLUSION

If a telephone interview would be of assistance in advancing the prosecution of the subject application, Applicants' undersigned attorney invites the Examiner to telephone her at the number provided below.

No fee, other than the fee for extension of time, is deemed necessary in connection with the filing of this response. Applicants hereby enclose a check in the amount of \$420.00 to cover the filing fees. If any further fee is necessary, the Patent Office is authorized to charge any additional fee to Deposit Account No. 50-0306.

Respectfully submitted,

Sarah B. Adriano

Sarah B. Adriano
Registration No. 34,470
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley, et al.
Serial No. : 09/609,915 Examiner: Lorraine Spector, Ph.D.
Filed : July 3, 2000 Group Art Unit: 1647
For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES
THEREOF

55 South Lake Ave., Suite 710
Pasadena, California 91101
August 2, 2004

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Madam/Sir:

**SUPPLEMENTAL AMENDMENT AND
CHANGE OF ELECTION IN RESPONSE TO RESTRICTION REQUIREMENT**

This Amendment is submitted to supplement Applicants' Amendment mailed June 2, 2004.
The June 2, 2004 Amendment was timely filed. Accordingly, no fees are due in connection
with this Supplemental Amendment.

Please amend the subject application as follows:

In the claims:

In compliance with the practice guidelines for making amendments, Applicants present
all pending claims with status indicators and submit new claims 29-30.

1. (Withdrawn) A soluble CTLA4 mutant molecule having the extracellular domain
of CTLA4 which binds CD80 or CD86.

2. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region S25-R33 of CTLA4 of Figure 3 (SEQ ID NO:2, at positions 51-59), and wherein the mutation is a substitution of any amino acid beginning with serine at position +25 of Figure 3 (SEQ ID NO:2, at position 51) and ending with arginine at position +33 of Figure 3 (SEQ ID NO:2, at position 59) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.
3. (cancelled)
4. (cancelled)
5. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region E95-G107 of CTLA4 of Figure 3 (SEQ ID NO:2, at positions 121-133), and wherein the mutation is a substitution of any amino acid beginning with glutamic acid at position +95 of Figure 3 (SEQ ID NO:2, at position 121) and ending with glycine at position +107 of Figure 3 (SEQ ID NO:2, at position 133) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.
6. (cancelled)
7. (cancelled)

8. (cancelled)

9. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region N108-I115 of CTLA4 of Figure 3 (SEQ ID NO:2, at positions 134-141), and wherein the mutation is a substitution of any amino acid beginning with asparagine at position +108 of Figure 3 (SEQ ID NO:2, at position 134) and ending with isoleucine at position +115 of Figure 3 (SEQ ID NO:2, at position 141) with a different amino acid selected from a group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

10. (cancelled)

11. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1 further comprising an amino acid sequence which alters the solubility, affinity or valency of the soluble CTLA4 mutant molecule for binding to CD80 or CD86.

12. (Withdrawn) The soluble CTLA4 mutant molecule of claim 11, wherein the amino acid sequence comprises a human immunoglobulin constant region.

13. (Withdrawn) A nucleic acid molecule comprising a nucleotide sequence encoding the amino acid sequence corresponding to the soluble CTLA4 mutant molecule of claim 1.

14. (Withdrawn) A vector comprising the nucleotide sequence of claim 13.

15. (Withdrawn) A host vector system comprising the vector of claim 14 in a suitable host cell.
16. (Withdrawn) The host vector system of claim 15, wherein the suitable host cell is a prokaryotic cell or a eukaryotic cell.
17. (Withdrawn) A method for producing a soluble CTLA4 mutant protein comprising growing the host vector system of claim 16 so as to produce the protein in the host cell and recovering the protein so produced.
18. (Withdrawn) A soluble CTLA mutant protein produced by the method of claim 17.
19. (Withdrawn) A method for regulating a T cell interaction with a CD80 and/or CD86 positive cell comprising contacting the CD80 and/or CD86 positive cell with the soluble CTLA4 mutant molecule of claim 1 so as to regulate the T cell interaction.
20. (Withdrawn) The method of claim 19, wherein the soluble CTLA4 mutant molecule is any of L104EA29L (SEQ ID NO.: 16), L104EA29T (SEQ ID NO.: 18), or L104EA29W (SEQ ID NO.: 20).
21. (Withdrawn) The method of claim 19, wherein the CD80 and/or CD86 positive cell is an antigen presenting cell.
22. (Withdrawn) The method of claim 19, wherein the interaction of the CTLA4-positive T cells with the CD80 and CD86 positive cells is inhibited.
23. (Withdrawn) A method for treating immunoproliferative diseases mediated by T cell interactions with B7 positive cells comprising administering to a subject the

soluble CTLA4 mutant molecule of claim 1, in an amount effective to regulate T cell interactions with said B7 positive cells.

24. (Withdrawn) The method of claim 23, wherein said T cell interactions are inhibited.
25. (Withdrawn) The method of claim 23, wherein the immunoproliferative disease is graft versus host disease.
26. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises amino acid residues from about position 93 to about position 124 of Figure 3 (SEQ ID NO:2 beginning at position 119 and ending at position 150) joined to amino acid residues from about position 1 to about position 94 of the amino acid sequence corresponding to the extracellular domain of CD28.
27. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein in the extracellular domain of CTLA4, the second proline in the amino acid motif MYPPPY (SEQ ID NO:35) is replaced with alanine.
28. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises:
 - a. one or more mutations in a region S25-R33 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 51-59), and wherein the mutation is a substitution of any amino acid beginning with serine at position +25 of Figure 3 (SEQ ID NO.: 2, at position 51) and ending with arginine at position +33 of Figure 3 (SEQ ID NO.: 2, at position 59) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine,

methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine; and

- b. one or more mutations in a region E95-G107 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 121-133), and wherein the mutation is a substitution of any amino acid beginning with glutamic acid at position +95 of Figure 3 (SEQ ID NO.: 2, at position 121) and ending with glycine at position +107 of Figure 3 (SEQ ID NO.: 2, at position 133) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

Please add new claims 29-30 as follows:

- 29. (New) A CTLA4Ig fusion protein reactive with B7 antigen and encoded by DNA deposited as ATCC 68629.--
- 30. (New) A CTLA4Ig fusion protein having the amino acid sequence of a CTLA4Ig fusion protein expressed by the cell deposited as ATCC 10762, wherein said CTLA4Ig fusion protein is reactive with B7 antigen.

REMARKS

Claims 1-2, 5, 9, 11-18 and 26-28 were pending and subject to restriction. New claims 29-30 have been added. Accordingly, claims 1-2, 5, 9, 11-18 and 26-30 are pending but only claims 29-30 are being examined.

Support for new claim 29 may be found in the specifications of:

1. U.S. Serial No. 07/723,617, filed June 27, 1991, at page 8, lines 10-15; page 11, lines 31-35.
2. U.S. Serial No. 08/008,898, filed January 22, 1993 (now U.S. Patent No. 5,770,197), at page 7, lines 20-22; page 17, lines 1-5.
3. U.S. Serial No. 08/228,208, filed April 15, 1994 (now U.S. Patent No. 6,090,914), at page 8, lines 1-5; page 10, lines 1-3 and page 19, lines 1-5.
4. U.S. Serial No. 09/609,915, filed June 3, 2000 (subject application), at page 3, line 3; page 11, lines 1-4; page 34, lines 9-11.

Support for new claim 30 is found in the specifications of:

1. U.S. Serial No. 07/723,617, filed June 27, 1991, at page 8, lines 10-15; page 18, lines 22-26.
2. U.S. Serial No. 08/008,898, filed January 22, 1993 (now U.S. Patent No. 5,770,197), at page 7, lines 20-22; page 23, lines 25-29.
3. U.S. Serial No. 08/228,208, filed April 15, 1994 (now U.S. Patent No. 6,090,914), at page 8, lines 1-5; page 10, lines 1-3; page 25, lines 25-29.
4. U.S. Serial No. 09/609,915, filed June 3, 2000 (subject application), at page 3, line 3; page 11, lines 4-6; page 34, lines 18-19.

Accordingly, new claims 29-30 do not introduce new matter, and their entry is respectfully requested.

Applicants: Peter S. Linsley, et al.
U.S. Serial No. 09/609,915
Filed: July 3, 2000
Page: 8

RESTRICTION REQUIREMENT AND ELECTION WITH TRAVERSE

On November 4, 2002, Applicants elected, with traverse, the invention of Group I in response to a restriction requirement dated October 2, 2002. On June 2, 2004, Applicants elected the species of claim 26 in response to a Species Election Requirement dated March 2, 2004.

Applicants hereby add new claims 29-30 which belong to newly created Group IV.

Applicants hereby change their previous election, of the invention of Group I, in response to the restriction requirement, and instead, now elect, with traverse, the invention of Group IV, as embodied in new claims 29-30 hereinabove. Accordingly, claims 1-2, 5, 9, 11-18 and 26-28 are withdrawn and only claims 29-30 are being examined.

CONCLUSION

If a telephone interview would be of assistance in advancing the prosecution of the subject application, Applicants' undersigned attorney invites the Examiner to telephone her at the number provided below.

Applicants: Peter S. Linsley, et al.
U.S. Serial No. 09/609,915
Filed: July 3, 2000
Page: 9

No fee is deemed necessary in connection with the filing of this response. If any further fee is necessary, the Patent Office is authorized to charge any additional fee to Deposit Account No. 50-0306.

Respectfully submitted,



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Registration No. 34,470
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Peter S. Linsley, et al.
Serial No. : 09/609,915 Examiner: Lorraine Spector, Ph.D.
Filed : July 3, 2000 Group Art Unit: 1647
For : SOLUBLE CTLA4 MUTANT MOLECULES AND USES
THEREOF

55 South Lake Ave., Suite 710
Pasadena, California 91101
August 23, 2004

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Madam/Sir:

**AMENDMENT IN RESPONSE TO
NOTICE OF NON-COMPLIANT AMENDMENT UNDER 37 C.F.R. §1.121**

This Amendment is being submitted in response to the Notice of Non-Compliant Amendment, dated August 9, 2004, issued by the U.S. Patent and Trademark Office in connection with the above-identified patent application. A response to the Notice is due September 9, 2004. Accordingly, this Amendment is being timely filed.

Please amend the subject application as follows:

In the claims:

In compliance with the practice guidelines for making amendments, Applicants present all pending claims with status indicators and submit new claims 29-30.

1. (Withdrawn) A soluble CTLA4 mutant molecule having the extracellular domain of CTLA4 which binds CD80 or CD86.
2. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region S25-R33 of CTLA4 of Figure 3 (SEQ ID NO:2, at positions 51-59), and wherein the mutation is a substitution of any amino acid beginning with serine at position +25 of Figure 3 (SEQ ID NO:2, at position 51) and ending with arginine at position +33 of Figure 3 (SEQ ID NO:2, at position 59) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.
3. (cancelled)
4. (cancelled)
5. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region E95-G107 of CTLA4 of Figure 3 (SEQ ID NO:2, at positions 121-133), and wherein the mutation is a substitution of any amino acid beginning with glutamic acid at position +95 of Figure 3 (SEQ ID NO:2, at position 121) and ending with glycine

at position +107 of Figure 3 (SEQ ID NO:2, at position 133) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

6. (cancelled)

7. (cancelled)

8. (cancelled)

9. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region N108-I115 of CTLA4 of Figure 3 (SEQ ID NO:2, at positions 134-141), and wherein the mutation is a substitution of any amino acid beginning with asparagine at position +108 of Figure 3 (SEQ ID NO:2, at position 134) and ending with isoleucine at position +115 of Figure 3 (SEQ ID NO:2, at position 141) with a different amino acid selected from a group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

10. (cancelled)

11. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1 further comprising an amino acid sequence which alters the solubility, affinity or valency of the soluble CTLA4 mutant molecule for binding to CD80 or CD86.

12. (Withdrawn) The soluble CTLA4 mutant molecule of claim 11, wherein the amino acid sequence comprises a human immunoglobulin constant region.
13. (Withdrawn) A nucleic acid molecule comprising a nucleotide sequence encoding the amino acid sequence corresponding to the soluble CTLA4 mutant molecule of claim 1.
14. (Withdrawn) A vector comprising the nucleotide sequence of claim 13.
15. (Withdrawn) A host vector system comprising the vector of claim 14 in a suitable host cell.
16. (Withdrawn) The host vector system of claim 15, wherein the suitable host cell is a prokaryotic cell or a eukaryotic cell.
17. (Withdrawn) A method for producing a soluble CTLA4 mutant protein comprising growing the host vector system of claim 16 so as to produce the protein in the host cell and recovering the protein so produced.
18. (Withdrawn) A soluble CTLA mutant protein produced by the method of claim 17.
19. (Withdrawn) A method for regulating a T cell interaction with a CD80 and/or CD86 positive cell comprising contacting the CD80 and/or CD86 positive cell with the soluble CTLA4 mutant molecule of claim 1 so as to regulate the T cell interaction.
20. (Withdrawn) The method of claim 19, wherein the soluble CTLA4 mutant molecule is any of L104EA29L (SEQ ID NO.: 16), L104EA29T (SEQ ID NO.: 18), or L104EA29W (SEQ ID NO.: 20).

21. (Withdrawn) The method of claim 19, wherein the CD80 and/or CD86 positive cell is an antigen presenting cell.
22. (Withdrawn) The method of claim 19, wherein the interaction of the CTLA4-positive T cells with the CD80 and CD86 positive cells is inhibited.
23. (Withdrawn) A method for treating immunoproliferative diseases mediated by T cell interactions with B7 positive cells comprising administering to a subject the soluble CTLA4 mutant molecule of claim 1, in an amount effective to regulate T cell interactions with said B7 positive cells.
24. (Withdrawn) The method of claim 23, wherein said T cell interactions are inhibited.
25. (Withdrawn) The method of claim 23, wherein the immunoproliferative disease is graft versus host disease.
26. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises amino acid residues from about position 93 to about position 124 of Figure 3 (SEQ ID NO:2 beginning at position 119 and ending at position 150) joined to amino acid residues from about position 1 to about position 94 of the amino acid sequence corresponding to the extracellular domain of CD28.
27. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein in the extracellular domain of CTLA4, the second proline in the amino acid motif MYPPPY (SEQ ID NO:35) is replaced with alanine.

28. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises:
- a. one or more mutations in a region S25-R33 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 51-59), and wherein the mutation is a substitution of any amino acid beginning with serine at position +25 of Figure 3 (SEQ ID NO.: 2, at position 51) and ending with arginine at position +33 of Figure 3 (SEQ ID NO.: 2, at position 59) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine; and
 - b. one or more mutations in a region E95-G107 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 121-133), and wherein the mutation is a substitution of any amino acid beginning with glutamic acid at position +95 of Figure 3 (SEQ ID NO.: 2, at position 121) and ending with glycine at position +107 of Figure 3 (SEQ ID NO.: 2, at position 133) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

Please add new claims 29-30 as follows:

- 29. (New) A CTLA4Ig fusion protein reactive with B7 antigen and encoded by DNA deposited as ATCC 68629.--
- 30. (New) A CTLA4Ig fusion protein having the amino acid sequence of a CTLA4Ig fusion protein expressed by the cell deposited as ATCC 10762, wherein said CTLA4Ig fusion protein is reactive with B7 antigen.

Applicants: Peter S. Linsley, et al.
U.S. Serial No. 09/609,915
Filed: July 3, 2000
Page: 7

REMARKS

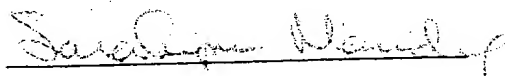
Claims 1-2, 5, 9, 11-18 and 26-28 were pending and subject to restriction. New claims 29-30 have been added. Accordingly, claims 1-2, 5, 9, 11-18 and 26-30 are pending but only claims 29-30 are being examined.

In the August 9 Notice, the Patent Office states that the response previously filed on August 2, 2004, is non-compliant because the claim amendments should start on a separate page. In response, Applicants have amended this paper to include claim amendments that begin on a separate page.

In view of Applicants' Amendment dated August 2, 2004 and this response, Applicants respectfully request reconsideration of all outstanding rejections and objections to the present application and allowance of the claims.

No fee is deemed necessary in connection with the filing of this response. If any further fee is necessary, the Patent Office is authorized to charge any additional fee to Deposit Account No. 50-0306.

Respectfully submitted,



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30436.30US12

PENDING CLAIMS

AS OF 8/2/04 (RESUBMITTED ON 8/23/04)

Submitted as Supplemental Amendment and Change of Election in Response to
Restriction Requirement

1. (Withdrawn) A soluble CTLA4 mutant molecule having the extracellular domain of CTLA4 which binds CD80 or CD86.
2. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region S25-R33 of CTLA4 of Figure 3 (SEQ ID NO:2, at positions 51-59), and wherein the mutation is a substitution of any amino acid beginning with serine at position +25 of Figure 3 (SEQ ID NO:2, at position 51) and ending with arginine at position +33 of Figure 3 (SEQ ID NO:2, at position 59) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.
- 3-4. (cancelled)
5. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region E95-G107 of CTLA4 of Figure 3 (SEQ ID NO:2, at positions 121-133), and wherein the mutation is a substitution of any amino acid beginning with glutamic acid at position +95 of Figure 3 (SEQ ID NO:2, at position 121) and ending with glycine at position +107 of Figure 3 (SEQ ID NO:2, at position 133) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

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9. (Withdrawn) The soluble CTLA4 mutant molecule of claim 1, wherein the extracellular domain of CTLA4 comprises one or more mutations in a region N108-I115 of CTLA4 of Figure 3 (SEQ ID NO:2, at positions 134-141), and wherein the mutation is a substitution of any amino acid beginning with asparagine at position +108 of Figure 3 (SEQ ID NO:2, at position 134) and ending with isoleucine at position +115 of Figure 3 (SEQ ID NO:2, at position 141) with a different amino acid selected from a group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

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12. (Withdrawn) The soluble CTLA4 mutant molecule of claim 11, wherein the amino acid sequence comprises a human immunoglobulin constant region.

13. (Withdrawn) A nucleic acid molecule comprising a nucleotide sequence encoding the amino acid sequence corresponding to the soluble CTLA4 mutant molecule of claim 1.

14. (Withdrawn) A vector comprising the nucleotide sequence of claim 13.

15. (Withdrawn) A host vector system comprising the vector of claim 14 in a suitable host cell.

16. (Withdrawn) The host vector system of claim 15, wherein the suitable host cell is a prokaryotic cell or a eukaryotic cell.
17. (Withdrawn) A method for producing a soluble CTLA4 mutant protein comprising growing the host vector system of claim 16 so as to produce the protein in the host cell and recovering the protein so produced.
18. (Withdrawn) A soluble CTLA mutant protein produced by the method of claim 17.
19. (Withdrawn) A method for regulating a T cell interaction with a CD80 and/or CD86 positive cell comprising contacting the CD80 and/or CD86 positive cell with the soluble CTLA4 mutant molecule of claim 1 so as to regulate the T cell interaction.
20. (Withdrawn) The method of claim 19, wherein the soluble CTLA4 mutant molecule is any of L104EA29L (SEQ ID NO.: 16), L104EA29T (SEQ ID NO.: 18), or L104EA29W (SEQ ID NO.: 20).
21. (Withdrawn) The method of claim 19, wherein the CD80 and/or CD86 positive cell is an antigen presenting cell.
22. (Withdrawn) The method of claim 19, wherein the interaction of the CTLA4-positive T cells with the CD80 and CD86 positive cells is inhibited.
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24. (Withdrawn) The method of claim 23, wherein said T cell interactions are inhibited.

25. (Withdrawn) The method of claim 23, wherein the immunoproliferative disease is graft versus host disease.
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- a. one or more mutations in a region S25-R33 of CTLA4 of Figure 3 (SEQ ID NO.: 2, at positions 51-59), and wherein the mutation is a substitution of any amino acid beginning with serine at position +25 of Figure 3 (SEQ ID NO: 2, at position 51) and ending with arginine at position +33 of Figure 3 (SEQ ID NO: 2, at position 59) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine; and
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at position +107 of Figure 3 (SEQ ID NO.: 2, at position 133) with a different amino acid selected from alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

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30. (New) A CTLA4Ig fusion protein having the amino acid sequence of a CTLA4Ig fusion protein expressed by the cell deposited as ATCC 10762, wherein said CTLA4Ig fusion protein is reactive with B7 antigen.

Richelle Domingo

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Paper No. 8

MANDEL & ADRIANO
35 N ARROYO PARKWAY
SUITE 60
PASADENA, CA 91103

COPY MAILED

JAN 25 2002

In re Application of
Peter S. Linsley et al
Application No. 09/609,915
Filed: July 3, 2000
For: SOLUBLE CTLA4 MUTANT
MOLECULES AND USES THEREOF

OFFICE OF PETITIONS
:
:
DECISION GRANTING
STATUS UNDER 37 CFR 1.47(a)
:
:

This is a decision on the petition filed January 4, 2002, requesting reconsideration of a decision mailed September 5, 2001, which refused to accord 37 CFR 1.47(a) status to the above-identified application.

The petition is granted.

Petitioner has shown that the non-signing inventor has refused to join in the filing of the above-identified application.

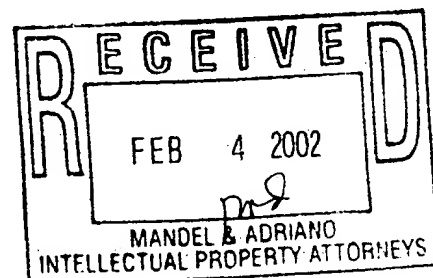
The above-identified application and papers have been reviewed and found in compliance with 37 CFR 1.47(a). This application is hereby accorded Rule 1.47(a) status. As provided in Rule 1.47(c), this Office will forward notice of this application's filing to the non-signing inventor at the address given in the petition. Notice of the filing of this application will also be published in the Official Gazette.

Telephone inquiries regarding this decision should be directed to Petitions Examiner Wan Laymon at (703) 306-5685.

This application is being forwarded to Technology Center AU 1646 for examination in due course.


Frances Hicks

Lead Petitions Examiner
Office of Petitions
Office of the Deputy Commissioner
for Patent Examination Policy



940
11



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Paper No. 9

JEFFREY A LEDBETTER
306 N.W. 113TH PLACE
SEATTLE, WA 98117

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JAN 25 2002

OFFICE OF PETITIONS

In re Application of
Linsley; Ledbetter; Bajorath; peach; Brady; Wallace; and Damle
Application No. 09/609,915
Filed: July 3, 2000
For: SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

Dear Mr. Ledbetter:

You are named as a joint inventor in the above identified United States patent application, filed under the provisions of 35 U.S.C. 116 (United States Code), and 37 CFR 1.47(a), Rules of Practice in Patent Cases. Should a patent be granted on the application you will be designated therein as a joint inventor.

As a named inventor you are entitled to inspect any paper in the file wrapper of the application, order copies of all or any part thereof (at a prepaid cost per 37 CFR 1.19) or make your position of record in the application. Alternatively, you may arrange to do any of the preceding through a registered patent attorney or agent presenting written authorization from you. If you care to join in the application, counsel of record (see below) would presumably assist you. Joining in the application would entail the filing of an appropriate oath or declaration by you pursuant to 37 CFR 1.63.

Telephone inquiries regarding this communication should be directed to Wan Laymon at (703) 306-5685. Requests for information regarding your application should be directed to the File Information Unit at (703) 308-2733. Information regarding how to pay for and order a copy of the application, or a specific paper in the application, should be directed to Certification Division at (703) 308-9726 or 1 (800) 972-6382 (outside the Washington D.C. area).

Frances Hicks
Frances Hicks

Lead Petitions Examiner
Office of Petitions
Office of the Deputy Commissioner
for Patent Examination Policy

CC:

MANDEL & ADRIANO
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SUITE 60
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